Chapter 12
Tetramisole and Levamisole Suppress Neuronal Activity Independently from Their Inhibitory Action on Tissue Non-specific Alkaline Phosphatase in Mouse Cortex

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Abstract Tissue non-specific alkaline phosphatase (TNAP) may be involved in the synthesis of GABA and adenosine, which are the main inhibitory neurotransmitters in cortex. We explored this putative TNAP function through electrophysiological recording (local field potential) in slices of mouse somatosensory cortex maintained in vitro. We used tetramisole, a well documented TNAP inhibitor, to block TNAP activity. We expected that inhibiting TNAP with tetramisole would lead to an increase of neuronal response amplitude, owing to a diminished availability of GABA and/or adenosine. Instead, we found that tetramisole *reduced* neuronal response amplitude in a dose-dependent manner. Tetramisole also decreased axonal conduction velocity. Levamisole had identical effects. Several control experiments demonstrated that these actions of tetramisole were independent from this compound acting on TNAP. In particular, tetramisole effects were not stereo-specific and they were not mimicked by another inhibitor of TNAP, MLS-0038949. The decrease of axonal conduction velocity and preliminary intracellular data suggest that tetramisole blocks voltage-dependent sodium channels. Our results imply that levamisole or tetramisole should not be used with the sole purpose of inhibiting TNAP in living excitable cells as it will also block all processes that are activity-dependent. Our data

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and a review of the literature indicate that tetramisole may have at least four different targets in the nervous system. We discuss these results with respect to the neurological side effects that were observed when levamisole and tetramisole were used for medical purposes, and that may recur nowadays due to the recent use of levamisole and tetramisole as cocaine adulterants.

Keywords Vitamin B6 · Pyridoxal · Ectonucleotidase · Epilepsy · Inflammatory leukoencephalopathy

12.1 Introduction

Alkaline phosphatases (APs) are a group of enzymes that release inorganic phosphate from phosphomonoesters. APs are ecto-enzymes, thus located on the extracellular side of the cells. Four types of APs have been identified in human. Three have restricted regional distributions and have been named accordingly as intestinal AP, germinal AP and placental AP. The fourth type, the tissue non-specific alkaline phosphatase (TNAP), has been found in all mammalian genera examined. As its name implies, TNAP shows a widespread distribution, being present in various tissues such as blood vessels, bone and cartilage, kidney, liver, lung, etc. (e.g., Borgers 1973; de Bernard et al. 1986; Morris et al. 1992; Hoshi et al. 1997).

In human, mutations of the TNAP gene lead to hypophosphatasia (Rathbun 1948), a rare disease characterized by low level of plasmatic AP and by abnormal bone mineralization (reviewed in: Fraser 1957; Millán 2006; Mornet 2007; Whyte 2010; Taketani et al. 2014; see also Chaps. 1 and 2 in this book). Importantly, the most severe forms of hypophosphatasia (perinatal and, to a lesser extent, infantile hypophosphatasia) are often associated with epileptic seizures (e.g., Rathbun 1948; Fraser 1957; Béthenod et al. 1967; Baumgartner-Sigl et al. 2007; Balasubramaniam et al. 2010; Taketani et al. 2014; see also Chaps. 14 and 15 in this book). In the mouse, inactivation of the TNAP gene produces an animal model of severe human hypophosphatasia, with bone mineralization defect and epileptic seizures as well (Waymire et al. 1995; Narisawa et al. 1997; see also Chap. 3).

Occurrence of epileptic seizures in TNAP KO mice and in the severe forms of human hypophosphatasia suggests that TNAP plays a fundamental role in the control of nerve cell activity—although indirect effects cannot be ruled out. This implies that TNAP should be present in the nervous tissue. Indeed, although early studies (Landow et al. 1942; Bourne 1943) did not report AP activity at the neuronal level in mammals—except in the spinal cord and medulla –, later studies demonstrated significant AP activity in multiple brain structures of various mammalian species. The pharmacological profile of this neuronal AP activity suggested it resulted from TNAP activity (Fonta et al. 2004; Langer et al. 2008) and it has recently been formally ascribed to TNAP gene expression (Ermonval et al. 2009; Brun-Heath et al. 2011).

AP activity in the brain is not homogenous; some structures show strong activity —hypothalamus, superior colliculus, subtancia nigra, spinal cord for example— while other—hippocampus, cerebellum, caudate nucleus, putamen for example— show much weaker activity (Shimizu 1950; Nandy and Bourne 1963; Friede 1966; Sugimura and Mizutani 1979; Langer et al. 2008; Brun-Heath et al. 2011; Street et al. 2013). AP activity is also observed in the cerebral cortex. In primate its level is particularly high in layer 4 of primary visual, auditory and somatosensory cortices (Friede 1966; Fonta and Imbert 2002; Fonta et al. 2004, 2005; Négyessy et al. 2011). In area 17 of primates, the thalamorecipient layers 4Cα and 4Cβ are exquisitely delineated by TNAP activity (Fonta and Imbert 2002; Fonta et al. 2004). In visual cortex, AP activity depends on neuronal activity, as revealed by monocular deprivation experiments (Fonta et al. 2004). TNAP is also highly expressed in the prefrontal cortex of primate (Fonta et al. 2004) and is found preferentially in layer 5 in non-sensory cortices of non-human primates (Friede 1966) and in human (Négyessy et al. 2011). AP activity is less patterned in rodent cortex but clear differences between cortical areas are nevertheless noticeable. For example, layer Ia of the piriform cortex and layer 4 of the somatosensory cortex show stronger AP activity than neighboring layers and surrounding cortical areas (Fonta et al. 2004; Langer et al. 2008; Brun-Heath et al. 2011).

In both cortical and subcortical structures, the suggestion that AP is located on synapses (Nandy and Bourne 1963) has been confirmed by electron microscopy studies, which revealed AP in the synaptic clefts of both excitatory and inhibitory synapses (Sugimura and Mizutani 1979; Mori and Nagano 1985; Fonta et al. 2004, 2005; Hanics et al. 2012; see also Chap. 5, this book). Strong AP activity has also been revealed on the nodes of Ranvier of myelinated axons (Pinner et al. 1964; Mori and Nagano 1985; Fonta et al. 2005; Hanics et al. 2012; see Chap. 5). Altogether, these data suggest that TNAP may be involved in the control of action potential propagation in axons as well as in the control of synaptic transmission.

In this study we used an electrophysiological approach with the aim to determine whether and how TNAP controls neuronal activity in the cerebral cortex, in particular through its putative involvement in the synthesis of two major neurotransmitters, GABA and adenosine. In cortex, both GABA and adenosine are inhibitory neurotransmitters: GABA acts postsynaptically through $GABA_A$ and $GABA_B$ receptors and presynaptically through $GABA_B$ receptors (e.g., Howe et al. 1987a, b; Connors et al. 1988; Deisz and Prince 1989; McCormick 1989). Adenosine mostly acts presynaptically through adenosine A1 receptors (e.g., Collins and Anson 1985; Fontanez and Porter 2006).

GABA is synthesized from glutamate by the glutamic acid decarboxylase (GAD) (e.g., Martin and Rimvall 1993). GAD uses the active form of vitamin B6, pyridoxal phosphate (PLP), as cofactor.[1] PLP cannot cross membranes; only the

[1]Note that GAD is one among multiple PLP-dependent enzymes. Approximately 60 genes encoding for enzymes using PLP as cofactor have been identified in mammals (Percudani and Peracchi 2009). In addition to GAD, some of these enzymes are directly involved in the synthesis of other neurotransmitters such as dopamine and serotonin (Ermonval et al. 2009).

nonphosphorylated form, pyridoxal (PL), can diffuse passively through membranes (e.g., Rifkin et al. 1972; Mehansho and Henderson 1980; see also Chap. 11, this book). TNAP plays a key role at this level as it is responsible for the dephosphorylation of extracellular PLP. In support for the involvement of TNAP in this process, the PLP concentration is markedly elevated in the serum of hypophosphatasic patients (Whyte et al. 1985, 1988). Likewise, PLP concentration in the serum of TNAP KO mice appears to be 20 time higher than in wild type mice (Waymire et al. 1995). In parallel, GABA levels appear to be strongly reduced in the brain of TNAP KO mice (Waymire et al. 1995; Fonta et al. 2012), confirming the importance of TNAP in the control of GABA synthesis. Yet the presence of AP on excitatory synapses suggests that its role is not solely limited to the control of GABA synthesis.

Adenosine synthesis in the extracellular space results from the degradation of ATP. ATP is released by neurons and glial cells (Fields and Burnstock 2006; Abbracchio et al. 2009; Butt 2011). ATP and intermediate nucleotides are rapidly hydrolyzed by a variety of ectonucleotidases. Four different ectonucleotidase families have been identified in the brain (e.g., Zimmermann et al. 2012). The first three families are quite specific: ectonucleoside triphosphate diphosphohydrolase degrades ATP in ADP and ADP in AMP; ectonucleotide pyrophosphatase/phosphodiesterase degrades ATP in AMP; and ecto-5′-nucleotidase degrades AMP in adenosine. In contrast, TNAP shows broader substrate specificity and is capable of degrading ATP to ADP, ADP to AMP, and AMP to adenosine. The ectonucleotidase activity of TNAP has been demonstrated in various tissues such as bone (e.g., Ciancaglini et al. 2010; Simão et al. 2013) and airways (Picher et al. 2003). Supports in favor of a role of TNAP in adenosine synthesis in the brain come from a cultured neuronal cell line study (Ohkubo et al. 2000) and from a recent spinal cord study (Street et al. 2013; see Chap. 13). Yet another study failed to reveal a significant role for TNAP in extracellular adenosine synthesis in the hippocampus (Zhang et al. 2012).

In order to examine the role of TNAP in the control of inhibitory synaptic transmission, we performed electrophysiological experiments on slices of mouse somatosensory cortex maintained in vitro. We used tetramisole to inhibit TNAP activity. Tetramisole is the racemic mixture of two stereoisomers: a levorotatory enantiomer, “levamisole”, and a dextrorotary enantiomer, “dexamisole”. Studies in the seventies showed that levamisole is highly effective at inhibiting TNAP (Van Belle 1972, 1976a, b) while dexamisole has no significant effect on this enzyme (Van Belle 1972, 1976b; Borgers 1973). Levamisole and tetramisole have since been used as TNAP inhibitors in a countless number of studies.

Our hypothesis was that suppressing TNAP activity with tetramisole would result in an *increase* of neuronal response amplitude owing to a reduction of pre- and/or postsynaptic inhibition mediated by GABA and/or adenosine. The results we obtained were opposite to this expectation: tetramisole, at concentration that fully suppress TNAP activity in biochemical assays (1–5 mM), also strongly *suppressed* neuronal activity. Levamisole had a similar effect. This suppression largely resulted from a reduction of action potential transmission along the axons. These results might have been interpreted as revealing a hitherto non-described control of axonal

excitability by TNAP present on the nodes of Ranvier, yet several control experiments revealed that neuronal activity suppression by tetramisole and levamisole was *not due to TNAP inhibition* by tetramisole and levamisole. Instead, we propose that tetramisole and levamisole block voltage-dependent sodium channels.

Our results therefore indicate that, in cortex, TNAP is not the unique target of tetramisole and levamisole. In addition, other neuronal processes are likely to be affected by levamisole and tetramisole: indeed, studies showed that, in the peripheral nervous system of mammals, levamisole and tetramisole may also interfere with adrenergic and cholinergic synaptic transmission.

Outside the nervous system, studies suggested that tetramisole and levamisole possess "immunostimulating" properties. For this reason, tetramisole and levamisole have received numerous clinical and pharmaceutical applications in a variety of diseases. Nevertheless, multiple side effects have been reported, including devastating neurological adverse effects such as epilepsy and inflammatory leukoencephalopathy, that have led to discontinue chronic tetramisole and levamisole usage in most countries. Yet these side effects have recently regained the attention of public health specialists because most of the illegal cocaine on the market nowadays appears to be adulterated with levamisole or tetramisole. We discuss the possible links between the neurological side effects of tetramisole and levamisole and the different targets of these compounds in the CNS.

12.2 Methods

12.2.1 Brain Slice Preparation

All procedures were conducted in accordance with the guidelines from the French Ministry of Agriculture (décret 87/848) and from the European Community (directive 86/609) and was approved by the local ethical committee (MP/06/79/11/12, comité d'éthique Midi-Pyrénées pour l'expérimentation animale). Adult (>2 month-old) wild type female mice were used for these experiments. Mouse was anesthetized with isoflurane. Once deeply anesthetized, the mouse was killed by decapitation. The scalp was removed, the skull was drilled, the upper part of the skull was lifted off, and the whole brain was carefully removed. These operations were performed in cold (3–4 °C) *modified* artificial cerebrospinal fluid (mACSF), whose composition was (in mM): NaCl 124, $NaHCO_3$ 26, KCl 3.5, $MgSO_4$ 1, $MgCl_2$ 9, NaH_2PO_4 1.25, and glucose 10. Note that Ca^{++} was omitted while Mg^{++} concentration was 10 mM. The rationale for using such a modified ACSF has been presented elsewhere (Nowak and Bullier 1996). The mACSF was oxygenated for 1 h before the beginning of the surgery with a mixture of 95 % O_2 and 5 % CO_2. Four hundred-micrometer-thick coronal brain slices were cut on a vibratome (752 M vibroslice, Campden Instrument, UK), whose chamber was filled with cold oxygenated mACSF. Once obtained, the slices were kept at room

temperature for at least one hour in a storage chamber filled with an in vivo-like (Brumberg et al. 2000; Sanchez-Vives and McCormick 2000) artificial cerebrospinal fluid (ACSF) of the following composition (in mM): NaCl 124, $NaHCO_3$ 26, KCl 3.5, $MgSO_4$ 1, NaH_2PO_4 1.25, $CaCl_2$ 1.2, and glucose 10. This ACSF was continuously bubbled with a 95 % O_2–5 % CO_2 mixture (pH 7.4). Recordings were performed in a submersion type chamber (Scientific System Design, Mississauga, Ontario, Canada) where the temperature was thermostatically held at 33–34 °C. The ACSF was gravity fed at a flow rate of 2.5–3.75 ml/min.

12.2.2 Recording and Stimulation

Neuronal signals examined in this study consisted mostly in local field potentials (LFPs) recorded in layer 4 and in the supragranular layers of the whisker, trunk and hindlimb representations of the primary somatosensory cortex (S1). Intracellular recordings were also attempted in these regions. In both cases, neuronal responses were evoked by extracellular electrical stimulation applied in the white matter or at the white matter-layer 6 border. Some extracellular recordings were also performed in the corpus callosum. In this later case, stimulation was applied in the corpus callosum too.

Tungsten-in-glass microelectrodes, with glass removed from the tip over a length of 45–110 μm, were used for extracellular electrical stimulation. Electrical stimulation was delivered through a stimulation isolation unit (A365 Stimulus Isolated, WPI) and consisted in monopolar, cathodal pulses of 0.2 or 0.3 ms duration. Pulses were delivered either in isolation at a frequency of 0.5 Hz, or as pair of pulses with an interstimulus interval of 20 ms, the pairs being repeated every 10 s (0.1 Hz). Stimulation intensity was between 50 and 180 μA.

LFPs were recorded through tungsten-in-glass microelectrodes and glass micropipettes. Glass micropipettes were pulled on a P97 Flaming Brown micropipette puller from 1.2 mm OD medium walled capillaries with filament (GC120F, Harvard Apparatus). The micropipette tip was broken to a 15–20 μm diameter opening and filled with ACSF (resistance 2–7 MΩ). The signal was amplified with an AxoClamp 2B amplifier (Axon Instrument, Foster City, CA) and further amplified with a Neurolog post-amplifier (final gain: × 1000). The signal was low-pass filtered at 10 kHz. Signals recorded through tungsten-in-glass microelectrodes (5–15 μm exposed tip) were amplified (×1000) and bandpass filtered (0.1 Hz–10 kHz) with the Neurolog recording system. Micropipettes for "sharp" intracellular recording were pulled on the P97 Flaming Brown micropipette puller from 1.2 mm OD medium walled capillaries with filament and filled with K-Acetate 3 M (DC resistance: 60–100 MΩ). The AxoClamp 2B was used for amplification (gain: × 10). The signal was low pass filtered (10 kHz). Signals were digitized with a 1401plus interface (CED, Cambridge, UK) with a digitization rate of 20 kHz.

12.2.3 Sample

The present study is based on a total of 27 successful experiments. By "experiment" we designate an ensemble of data obtained with one pair of stimulation and recording sites in one slice. Given the duration of the protocols used in this study (pharmacological manipulation requiring controls, several concentrations of various pharmacological compounds and recoveries), there was usually only one experiment per slice and per day, and by extension one experiment per mouse (19 experiments). More rarely two experiments were performed the same day on two distinct brain slices obtained from the same mouse, with one of the two experiments being dedicated to corpus callosum recording (8 experiments total).

12.2.4 Analysis

Signals were analyzed offline using spike2 software (CED) and custom scripts. The bulk of the data are issued from extracellular LFP recording evoked by electrical stimulation. The first pass of the LFP analysis consisted in averaging 6 or 30 consecutive sweeps, depending on stimulation frequency (0.1 or 0.5 Hz). This resulted in series of averaged LFPs for each consecutive minute of recording (Fig. 12.1d). These time series allowed following the time-course of the drug effects. They were visualized using Origin software (OriginLab Corporation, USA).

The averaged LFPs obtained in the gray matter (23 experiments) consisted in fast and slow components. The slow component, or field PSP (noted "fPSP" in Figures), reflects postsynaptic responses. Depending on where the recording electrode was located with respect to current sources and sinks (e.g., Mitzdorf 1985), the slow component appeared either as a slow positivity (Figs. 12.1e, 12.6a and 12.7a) or as a slow negativity (Fig. 12.5a). The fast components—always negative—correspond to *population spikes*, that is, to the synchronous discharge of action potentials by a population of neurons located in the vicinity of the recording electrode. Two types of population spikes were identified in our experiments: antidromic and orthodromic. *Antidromic* population spikes (indicated by "APS" in Figures) result from the (unnatural) retrograde propagation of action potentials elicited in the axons at the stimulation site toward the cell body, where spike firing is initiated *without* intercalated synapses. *Orthodromic* population spikes ("OPS" in Figures) are elicited when the (regular) propagation of action potentials along the axons results in postsynaptic responses that are large enough to trigger action potentials in the postsynaptic neurons (Fig. 12.1e, h). Since they are initiated by excitatory synaptic inputs, their latency is longer than that of antidromic responses. In addition, they disappear when synaptic transmission is suppressed. We systematically tested whether the population spikes we were recording from were orthodromic and/or antidromic by suppressing synaptic responses with ACSF solutions without Ca^{++} and containing either 6–10 mM Mg^{++} or 1.2–2 mM Mn^{++}. Antidromic population spikes were present in all but one of the

23 experiments involving gray matter recording, whereas measurable orthodromic responses occurred in 16 of these experiments.

The amplitude of antidromic population spikes was measured as the difference between the peak of the population spike and the pre-stimulus baseline. The latency corresponds to the latency of the peak of the population spikes relative to stimulus onset. As orthodromic population spike followed antidromic responses and were eventually partially merged with them, their amplitude was measured as the difference between the peak and the positivity corresponding to the repolarization of the preceding antidromic population spike. The amplitude of the slow component was measured relative to pre-stimulus baseline. When the orthodromic response showed both a slow component and a population spike, the amplitude of the response usually corresponds to that of the population spike. Population data in text corresponds to the mean ± SEM.

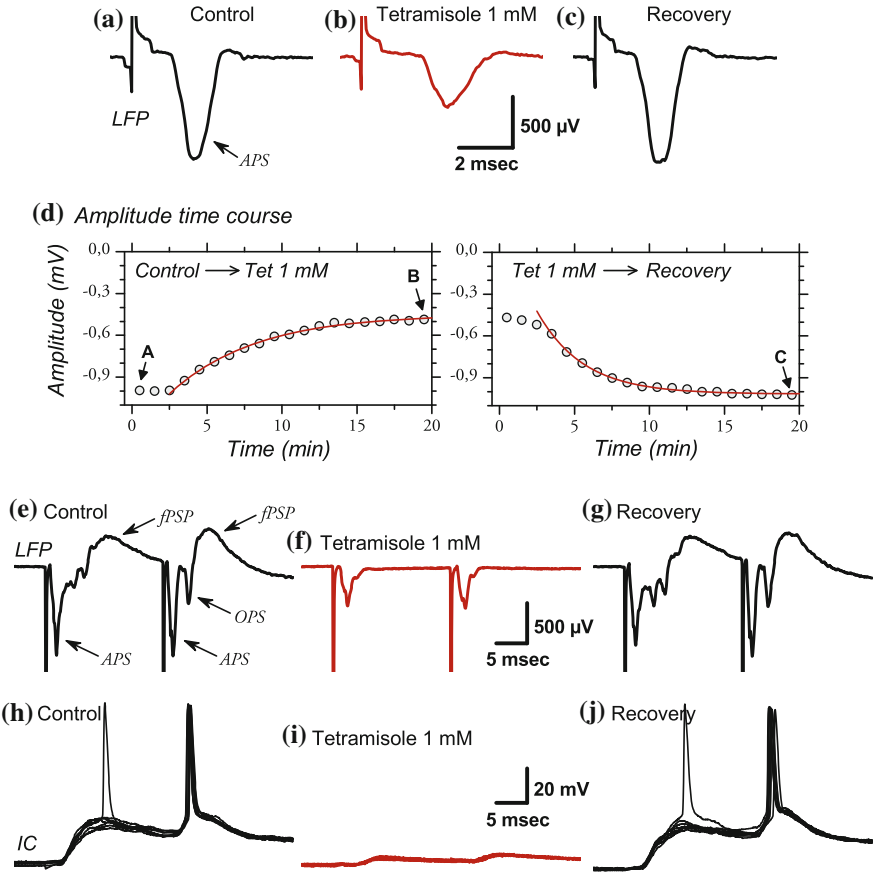
12.2.5 Chemicals

Tetramisole (0.1–5 mM), levamisole (0.5 and 1 mM), adenosine (100 μm), ATP (1 mM) and pyridoxal phosphate (10 μM) were dissolved in ACSF. MLS-0038949 (10 μM final concentration) was first dissolved in DMSO (final DMSO concentration: 0.1 % in ACSF). All chemicals were purchased from Sigma except MLS-0038949 purchased from Merck. When several concentrations of tetramisole or levamisole were used, their order of application was randomized from one experiment to the next.

12.3 Results

12.3.1 Tetramisole Reduces Both Orthodromic and Antidromic Responses in a Dose-Dependent Manner

Figure 12.1a–c illustrates the effects of levamisole (1 mM) on an antidromic population spike. Each trace corresponds to the average of 30 sweeps, representing 1 min of recording in control ACSF (Fig. 12.1a), 1 min of recording after 20 min of perfusion with ACSF containing 1 mM tetramisole (Fig. 12.1b) and 1 min of recording after 20 min of reperfusion with regular ACSF (recovery, Fig. 12.1c). The response was identified as antidromic as it persisted when Ca^{2+} was replaced by Mn^{2+} 2 mM (not shown). The white matter stimulation did not elicit a measurable orthodromic response in this experiment. In the presence of tetramisole (1 mM, Fig. 12.1b), the response amplitude was decreased to 49 % of the amplitude measured in control while the peak latency was increased from 2.6 to 3.5 ms. The amplitude and latency returned to near control values after tetramisole washout (Fig. 12.1c).



The time-course of the effect of tetramisole for the same experiment is depicted in Fig. 12.1d. The amplitude of the antidromic population spike is represented as a function of time when the control ACSF was replaced by ACSF + tetramisole 1 mM (left panel), and when ACSF + tetramisole 1 mM was replaced by control ACSF (right panel). Each data point represents the amplitude of the response measured in LFP traces averaged over 1 min. The curves are exponentials fitted to the data. The amplitude decreased upon perfusion of tetramisole 1 mM up to a plateau value that was reached after approximately 20 min. Recovery was complete in about 20 min as well.

The effect of tetramisole on orthodromic responses is illustrated with data from another experiment in Fig. 12.1e–g. Electrical stimulation was delivered in the form of pairs of pulses with an interstimulus interval of 20 ms. The LFP (Fig. 12.1e) consisted in an antidromic population spike (first population spike on traces, APS) and in orthodromic responses composed of a slow positivity (fPSP) and of an orthodromic population spike (second population spike on traces, OPS). The

◀ **Fig. 12.1** Both orthodromic and antidromic neuronal responses are reduced by tetramisole. **a–c** Effect of tetramisole 1 mM on an antidromic population spike (*APS*) recorded extracellularly (LFP) in the supragranular layers of mouse somatosensory cortex. Electrical stimulation in white matter (50 μA, 300 μsec, 0.5 Hz). Each trace is the average of 30 sweeps (1 min recording) obtained at the time of switching the perfusion from control ACSF to ACSF with tetramisole 1 mM (**a**), at the end of 20 min perfusion of ACSF with tetramisole 1 mM (**b**), and after 20 min perfusion with control *ACSF* (recovery, **c**). **a**, **b** Tetramisole reduced peak response amplitude from –1 to –0.49 mV and increased peak response latency from 2.6 to 3.5 ms. **c**: Effects of tetramisole 1 mM were reversible (amplitude: –1.03 mV, latency: 2.7 ms). **d** Time course of the effect of tetramisole on the amplitude of the *APS*. Same experiment as in **a–c**. Symbols correspond to the amplitude of the *APS* measured in *LFP* traces averaged over 1 min. Symbols indicated by letters **a–c** refers to the *LFP* traces displayed in **a–c**. *Solid lines* are exponential curves fitted to the data. The first 3 data points were not included in the fit as they approximately corresponded to the 3 min required for the replacement of one perfusion solution by the other in the recording chamber. *Left panel*: the time constant of amplitude decrease in tetramisole 1 mM was 5 min and 41 s. *Right panel*: the time constant of recovery in regular ACSF was 3 min and 4 s. The R^2 of fit was 0.99 for both fits. **e–g**: Effect of tetramisole 1 mM on APS, orthodromic population spike (OPS) and slow field PSP (fPSP) recorded extracellularly (LFP) in the supragranular layers of mouse somatosensory cortex. Electrical stimulation (80 μA, 200 μsec) was delivered in the white matter as pairs of pulses with an interval of 20 ms. Pairs of pulses delivered every 10 s (0.1 Hz). Traces in **e–g** correspond to the average of 6 sweeps (1 min of recording). **e** The first stimulation of the pair elicited an antidromic population spike (*APS*, –1.09 mV) followed by barely visible orthodromic spikes (not indicated) and by a slow positive component (fPSP). The second stimulation of the pair induced an *APS* whose amplitude (–1.10 mV) was identical to the one obtained after the first stimulation, followed by a well developed orthodromic population spike (OPS) and by a larger *fPSP*. The increase in amplitude of the orthodromic responses was due to paired-pulse facilitation. **f** Orthodromic responses (both OPS and fEPSP) were completely suppressed by tetramisole 1 mM and the antidromic response was reduced to –0.49 (first APS) or –0.51 (second APS) mV. **g** Both antidromic and orthodromic responses recovered upon return to control ACSF. APS amplitudes were –0.97 and –1.10 mV (first and second APS respectively). **h–j** Effect of tetramisole in an intracellularly recorded neuron. The intracellular recording (IC) was performed simultaneously with the LFP recording whose traces are displayed in **e–g**. Each panel shows 8 superimposed sweeps. The stimulation artifact was digitally removed. The white matter stimulation did not elicit an antidromic response in this neuron but it induced an EPSP whose amplitude, in control condition (**h**), was large enough to trigger orthodromic spikes, especially after the second pulse of the stimulus pair as a result of paired-pulse facilitation. **i** EPSP amplitude was drastically reduced by tetramisole (1 mM) and was too small to bring the membrane potential to firing threshold. **j** After tetramisole washout, EPSP amplitude came back to control value and reached spike firing threshold

orthodromic responses were more prominent after the second stimulation as a result of paired-pulse facilitation. In the presence of tetramisole (1 mM), the orthodromic responses were completely suppressed while the antidromic response was reduced to 45 % (first APS) or 46 % (second APS) of the control amplitude (Fig. 12.1f). The effect of tetramisole was reversible (Fig. 12.1g).

One intracellular recording was performed simultaneously with the extracellular LFP recording of Fig. 12.1e–g. In control ACSF, the intracellularly recorded response (Fig. 12.1h) consisted in an excitatory postsynaptic potential (EPSP) of 22 mV amplitude. The EPSP eventually triggered orthodromic action potentials after the first stimulation, and systematically did so after the second stimulation (Fig. 12.1h). The slow EPSP and fast action potentials offer a mirror image of the orthodromic responses recorded extracellularly (Fig. 12.1e). The postsynaptic

response was nearly completely suppressed by tetramisole 1 mM (Fig. 12.1i) and the peak of the remnant depolarization (4 mV) remained far below action potential threshold. Both responses recovered upon return to control ACSF (Fig. 12.1j). Since the resting membrane potential did not depolarize in the presence of tetramisole, the reduction of ortho- and antidromic response amplitude cannot be attributed to a depolarization block.

Figure 12.2a represents the effect of different concentrations of tetramisole on the antidromic population spike amplitude at the population level. Response amplitudes were normalized by the control response amplitude and are expressed as percentage of the control response amplitude. The reduction of the antidromic response amplitude was dose-dependent (ANOVA, $P < 0.0001$) and reversible. In comparison to control and recovery, response amplitude was significantly reduced with tetramisole at 0.5, 1, 2 and 5 mM (PLSD Fischer test, $P < 0.0001$ for all comparisons). Response suppression was nearly complete with 2 mM tetramisole (4.4 % ± 0.04 of control response amplitude).

Figure 12.2C shows the dose-response relationship for antidromic response reduction fitted with Hill's equation:

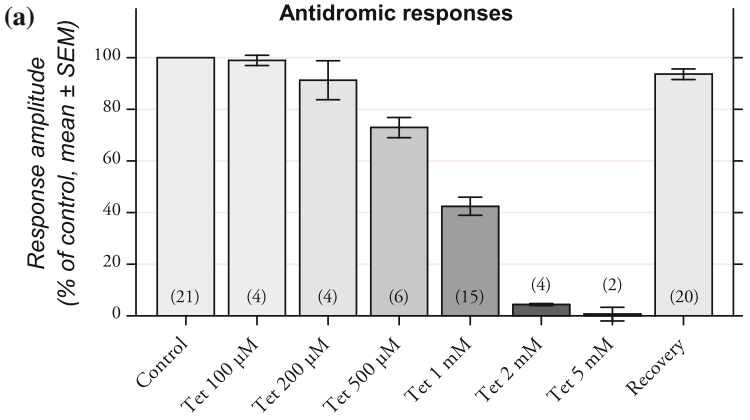
$$R = 100 - 100 \times \frac{C^n}{IC_{50}^n + C^n}$$

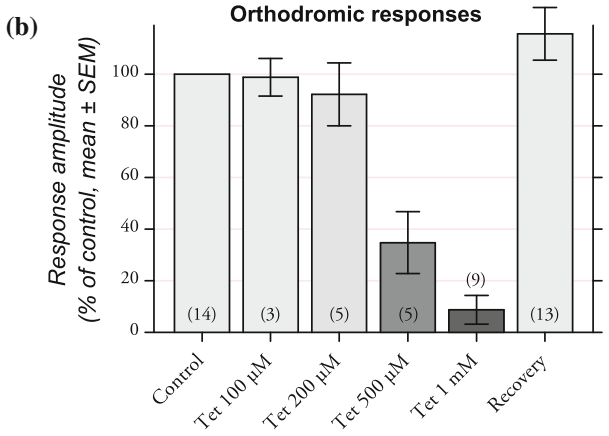
where R represent the normalized response amplitude (% of control), C the tetramisole concentration, IC_{50} the concentration producing half the maximal effect, and n the Hill coefficient determining the slope of the curve. The IC_{50} returned by the fit was 0.73 ± 0.08 (SE) mM and the Hill coefficient was 3.04 ± 0.38.

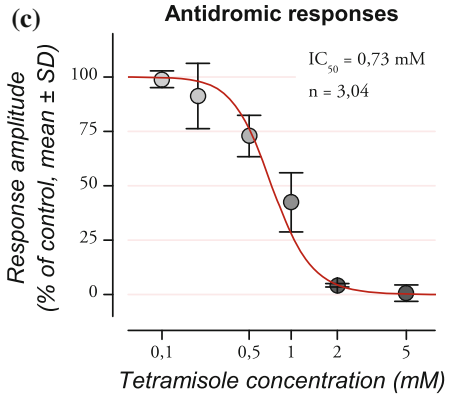
Tetramisole also reduced the amplitude of the orthodromic responses in a dose-dependent manner (ANOVA, $P < 0.0001$, Fig. 12.2b). The amplitude of orthodromic response was significantly less than control and recovery with tetramisole at 0.5 and 1 mM (PLSD Fischer test, $P < 0.0001$ for all comparisons).[2]

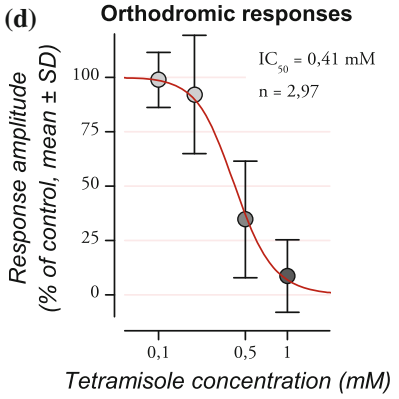
Comparing Fig. 12.2a, b, it can be seen that, with tetramisole at 0.5 and 1 mM, orthodromic responses were significantly more reduced than antidromic responses. With 0.5 mM the antidromic response amplitude represented 73 ± 4 % of the control whereas the orthodromic response was reduced to 35 ± 12 % of the control ($P = 0.01$, paired t-test); likewise, with 1 mM tetramisole the antidromic response amplitude (42 ± 4 % of control) was less reduced that the orthodromic response (9 ± 6 % of control) ($P = 0.0002$). The stronger effect of tetramisole on orthodromic response amplitude is confirmed by the Hill's equation fitted to the dose-response relationship, which returned an IC_{50} of 0.41 ± 0.15 mM (Fig. 12.2d). The Hill coefficient was 2.97 ± 2.27.

[2]Orthodromic responses could not be tested with 2 mM tetramisole: among the 4 experiments in which we used this concentration, 2 showed no measurable orthodromic response in the control condition, while in the other 2 experiments the orthodromic response obtained in the control condition failed to recover.


(a)
Antidromic responses
Response amplitude
(% of control, mean ± SEM)
100
80
60
40
20
0
(21)
(4)
(4)
(6)
(15)
(4)
(2)
(20)
Control
Tet 100 µM
Tet 200 µM
Tet 500 µM
Tet 1 mM
Tet 2 mM
Tet 5 mM
Recovery


(b)
Orthodromic responses
Response amplitude
(% of control, mean ± SEM)
100
80
60
40
20
0
(14)
(3)
(5)
(5)
(9)
(13)
Control
Tet 100 µM
Tet 200 µM
Tet 500 µM
Tet 1 mM
Recovery


(c)
Antidromic responses
Response amplitude
(% of control, mean ± SD)
IC50 = 0,73 mM
n = 3,04
100
75
50
25
0
0,1
0,5
1
2
5
Tetramisole concentration (mM)


(d)
Orthodromic responses
Response amplitude
(% of control, mean ± SD)
IC50 = 0,41 mM
n = 2,97
100
75
50
25
0
0,1
0,5
1
Tetramisole concentration (mM)

◀ **Fig. 12.2** Reduction of antidromic and orthodromic response amplitude by tetramisole is dose-dependent. **a–b** Bar graphs illustrating effect of various tetramisole ("Tet" on x-axis) concentrations (100 μM–5 mM) on antidromic (**a**) and orthodromic (**b**) response amplitude. Before pooling amplitudes were normalized by the control amplitudes and are expressed as a percentage of control response amplitude. Bar height represents the mean and error bars represent the SEM. Note near complete suppression of antidromic responses with tetramisole at 2 mM, and of orthodromic responses with tetramisole at 1 mM. In **a** and **b** the total number of tested concentrations is larger than the number of controls and recoveries because in some experiments several tetramisole concentrations were successively tested between one control and one recovery. For the discrepancy in the number of controls and recoveries in **a** and **b** see legend of Fig. 12.5. **c–d** Dose-response relationships for antidromic (**c**) and orthodromic (**d**) responses. The dots show mean normalized amplitudes, as in **a–b**, but the error bars represent 1 SD. The continuous lines correspond to the Hill equation (see Results) fitted to the data. The fits were weighted by the variance. The R^2 of the fits was 0.99 for both antidromic and orthodromic responses. The free parameters values (IC_{50} and n) are displayed in the figure

12.3.2 Tetramisole Increases Antidromic Response Latency

In addition to decreasing their amplitudes, tetramisole also increased the latency of the antidromic population spikes. This is illustrated with one example on Fig. 12.3a. In this experiment we applied 3 different tetramisole concentrations. It can be seen that both the onset latency and the latency to peak of the antidromic response increased with increasing concentration of tetramisole. Increase in latency is even visible with a tetramisole concentration of 0.5 mM although this concentration had a marginal effect on response amplitude in this experiment.

For population level analysis we examined and quantified the effect of tetramisole on the latency to peak of the antidromic population spikes. As shown in Fig. 12.3b, tetramisole had a clear, dose-dependent effect on peak latency (ANOVA, $P < 0.0001$). Relative to control (100 %), the latency was significantly increased (+11 ± 1.5 %) with 200 μM tetramisole (PLSD Fischer test, $P = 0.03$), although this concentration had no significant effect on response amplitude (Fig. 12.2a). Increases in latency were also significant with 0.5 mM (+17 ± 2 %, $P = 0.0004$), 1 mM (+41 ± 5 %, $P < 0.0001$) and 2 mM tetramisole; with 2 mM tetramisole, the latency was more than doubled (+110 ± 0.3 %, $P < 0.0001$). Thus, tetramisole not only reduced the amplitude of the responses, but also increased their latencies.

12.3.3 Tetramisole Reduces Amplitude and Increases Latency of Axonal *Population Spikes*

The increase of antidromic response latency could have two origins. The first is that tetramisole affected the somatic membrane potential, leading to an impairment of the coupling between the axon and the cell body. The second is that the increase in response latency originated from an action of tetramisole on the axons themselves.

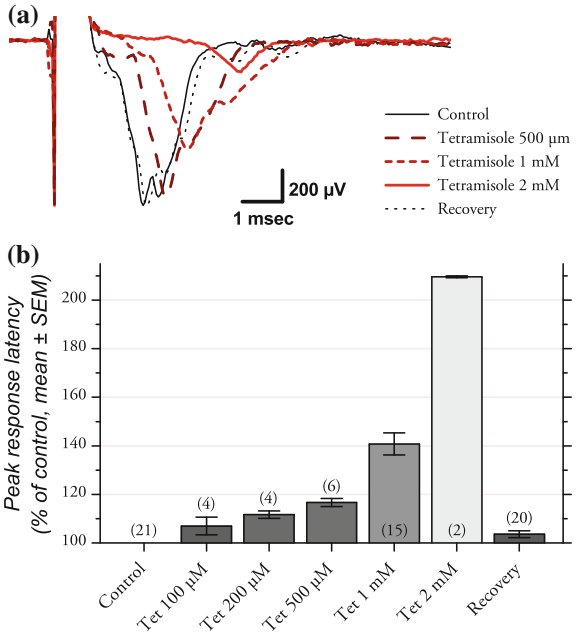


Fig. 12.3 Tetramisole increases antidromic response latency. **a** Effect of tetramisole at different concentrations on antidromic population spikes in one experiment. LFP recordings in the supragranular layers of the barrel cortex. Stimulation in the white matter (60 µA, 200 µsec). Each trace corresponds to the average of 30 sweeps (1 min recording) in control ACSF and in the presence of tetramisole at 0.5, 1 and 2 mM. Tetramisole decreased response amplitude and increased peak response latency (control: 2.33 ms; 0.5 mM: 2.86 ms; 1 mM: 3.47 ms; 2 mM: 4.78 ms; recovery: 2.42 ms). Onset latency also clearly increased in this experiment, indicating that increased peak latency was not simply due to increased spike duration. **b** Bar graph summarizing the effect of tetramisole ("Tet", 0.1–2 mM) on the peak latency of antidromic population spike at the population level. Effect of tetramisole at 2 mM has been examined in 4 experiments but the response was completely suppressed in two of these so that latency could not be measured. Before pooling latencies were normalized by the latency measured in control ACSF and are expressed as a percentage of the control latency. Bar height: mean; error bars: SEM

In order to distinguish between these two possibilities, we made experiments in which both stimulation and recording were performed in the corpus callosum. The corpus callosum is the major axonal tract of the central nervous system. It mostly contains myelinated axons. The electrical stimulation initiated *axonal* population spikes that traveled along the axons. The axonal population spikes were recorded at approximately 1 mm from the stimulating electrode. The example in Fig. 12.4a illustrates the effect of tetramisole at 1 mM: as for antidromic population spikes recorded in the cortical gray matter, the axonal population spike recorded in the white matter showed both reduced amplitude and increased latency. The effects of tetramisole (1 mM) averaged over 4 experiments are summarized in Fig. 12.4b: tetramisole significantly reduced the peak response amplitude to a value representing 56 ± 7 % of the control amplitude (paired t-test, $P = 0.008$) and the peak

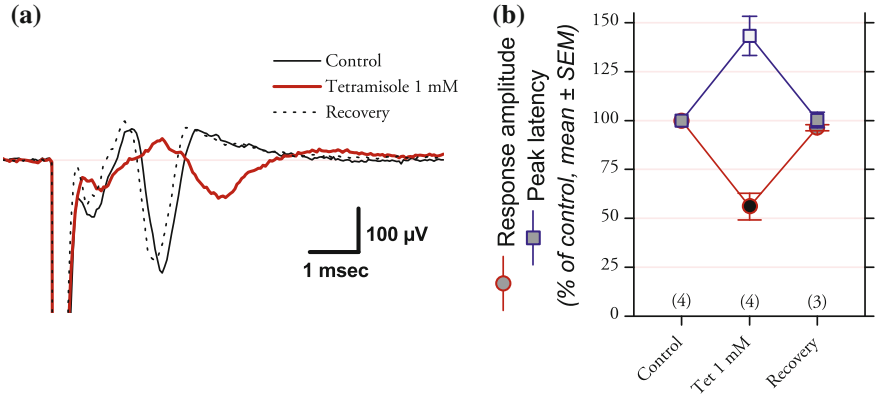


Fig. 12.4 Tetramisole reduces axonal population spike amplitude and reduces axonal conduction velocity. For these experiments both stimulation and recording were performed in the corpus callosum. **a** Example of the effect of tetramisole (1 mM) on axonal population spike amplitude and latency. Each trace corresponds to the average of 6 sweeps (1 min recording). Stimulation in the midline of the corpus callosum; recording approximately 1 mm lateral to the midline, below motor cortex. The amplitude of the axonal population spike was reduced in tetramisole 1 mM (control: –0.33 mV; tetramisole 1 mM: –0.14 mV; recovery: –0.32 mV). The peak latency was increased in tetramisole (control: 2.25 ms; tetramisole 1 mM: 3.54 ms; recovery: 2.10 ms), implying reduced conduction velocity in the axons. **b** Effects of tetramisole 1 mM ("Tet 1 mM") on the amplitude and latency of axonal population spikes, group data. Amplitudes and latencies normalized by control values. The squares represent the mean peak latency and the circles represent the mean amplitude; the error bars represent the SEM

latency was significantly increased by +43 ± 10 % relative to the control latency ($P = 0.02$, Fig. 12.4b). These data indicate that action potential transmission along the axons, hence axonal conduction velocity, was altered by tetramisole.

12.3.4 Effect of Tetramisole on Antidromic Population Spikes Is not Compensated by Pyridoxal

The data we have presented thus far suggest that orthodromic and antidromic response suppression by tetramisole results in large part from an impaired transmission of action potentials along the axons. Action potential electrogenesis in myelinated axons takes place at the node of Ranvier. Coincidentally, TNAP appears to be highly concentrated at the nodes of Ranvier. Since one of the main substrate of TNAP is PLP, we hypothesized that tetramisole, by preventing extracellular PLP to PL conversion, perturbed an (unknown to us) intracellular PL/PLP-dependent mechanism involved in action potential initiation and/or propagation. To test this possibility, we recorded antidromic population spikes and compared the effect of tetramisole 1 mM with the effect of tetramisole 1 mM to which we added PL, expecting that exogenous PL would compensate for the inhibition of TNAP by

tetramisole. We used a PL concentration of 10 µM, a value much larger than the concentration measured in the CSF (≤0.1 µM: Spector 1978, van der Ham et al. 2012). An example is presented in Fig. 12.5a. The effect of tetramisole alone is identical to that illustrated previously: a reduction of the antidromic population spike amplitude associated with an increased latency. Yet the trace obtained with tetramisole 1 mM + PL 10 µM is nearly identical to that obtained with tetramisole 1 mM alone, indicating that PL did not palliate the action of tetramisole. When examined at the population level (Fig. 12.5b), the mean population spike amplitude measured in the presence of tetramisole did not differ significantly from that

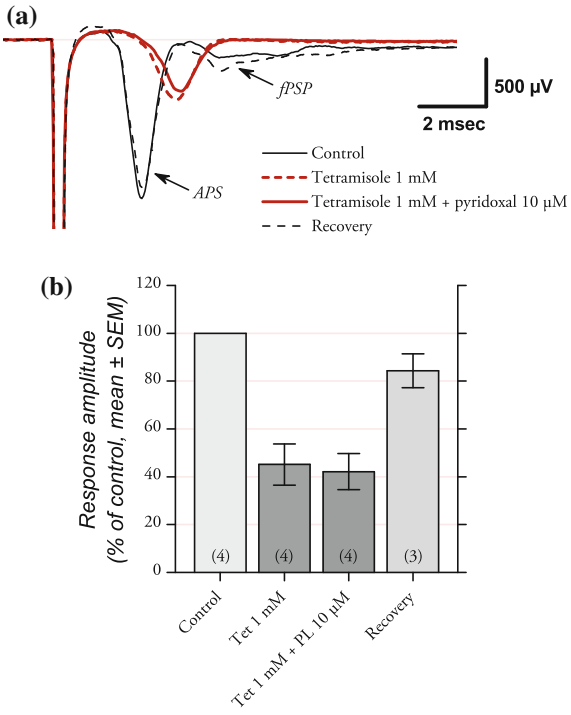


Fig. 12.5 Extracellular PL does not compensate for the effect of tetramisole. **a** Comparison of the effects of tetramisole at 1 mM and tetramisole 1 mM + pyridoxal 10 µM in one experiment. Recording in the supragranular layers of the somatosensory cortex, stimulation in the white matter. Each trace is the average of 30 sweeps (1 min recording) in control ACSF (control and recovery), in ASCF + tetramisole 1 mM, and in ACSF + tetramisole 1 mM + PL 10 µm. Tetramisole reduced the antidromic population spike amplitude and increased its latency, and completely suppressed the slow postsynaptic response (fPSP). PL did not compensate for the effect of tetramisole. **b** Bar graph summarizing the effect of tetramisole 1 mM or tetramisole 1 mM + PL 10 µM on antidromic population spike amplitude. As previously data were normalized by control response amplitude and are expressed as a percentage of control response amplitude. Bar height: mean; error bars: SEM. The discrepancy between the number of controls and recoveries comes from one experiment in which the order of drug application and tests was: control ACSF → tetramisole 1 mM → tetramisole 1 mM + PL 10 µM → tetramisole 1 mM. Response amplitude during the first and second application of tetramisole 1 mM were perfectly identical, and therefore fitted with our stationarity criteria although recovery was not tested in regular ACSF in this experiment

measured in the presence of tetramisole + PL (paired t-test, $P = 0.11$, n = 4 experiments). On the other hand, both were significantly less than the control response amplitude (tetramisole 1 mM alone: 45 ± 9 % of control, $P = 0.008$; tetramisole 1 mM + PL 10 μM: 42 ± 8 % of control, $P = 0.005$). In short, the decreased amplitude and increased latency of the antidromic responses cannot be explained by the alteration of a PL/PLP-dependent mechanism consecutive to TNAP inhibition by tetramisole.

12.3.5 Effect of Tetramisole Is not Compensated by Adenosine and Is not Mimicked by ATP

As outlined in the introduction, TNAP could be one of the ectonucleotidases involved in extracellular adenosine synthesis. Noticeably, a study (Irnich et al. 2002) suggested that adenosine receptors might control the excitability of peripheral nerves axons, although we are not aware of any study demonstrating that this also applies to cortical axons. Therefore, another possibility we examined was that the reduction of antidromic and axonal population spike amplitudes could be consecutive to a decrease in extracellular adenosine concentration due to TNAP inhibition by tetramisole. To explore this possibility, we compared the effect of tetramisole 1 mM alone with those of tetramisole 1 mM to which 100 μM of adenosine was added. This concentration of adenosine corresponds to the one that has maximal or near maximal effect on A1 receptors in cortex (Fontanez and Porter 2006). However, exogenous adenosine did not compensate for the effect of tetramisole. The example shown in Fig. 12.6a shows that the antidromic population spike in the presence of 1 mM tetramisole + 100 μM adenosine was barely distinguishable from that obtained in the presence of tetramisole alone. Both were strongly decreased in amplitude in comparison to control and recovery traces. On average (3 experiments, Fig. 12.6b), the antidromic population spike reduction with tetramisole 1 mM + adenosine 100 μM did not differ significantly from that observed with tetramisole 1 mM alone ($P = 0.09$, paired t-test, 52 ± 11 % vs. 40 ± 9 % of control response amplitude respectively). Both were significantly reduced in comparison to control ($P = 0.04$ and $P = 0.02$ respectively). Therefore, reduction of response amplitude in the presence of tetramisole does not seem to be explained by a reduced extracellular concentration of adenosine following TNAP inhibition by tetramisole.

Alternatively, blocking the ectonucleotidase activity of TNAP may also have led to an increased concentration of extracellular ATP. We therefore examined whether increasing extracellular ATP concentration mimicked the effect of tetramisole on antidromic population spikes (n = 2). As illustrated in Fig. 12.6c, extracellular ATP (1 mM) did not modify the amplitude or the latency of the antidromic responses. This suggests that the effect of tetramisole on antidromic population spikes is not the consequence of a heightened extracellular concentration of ATP due to TNAP inhibition.

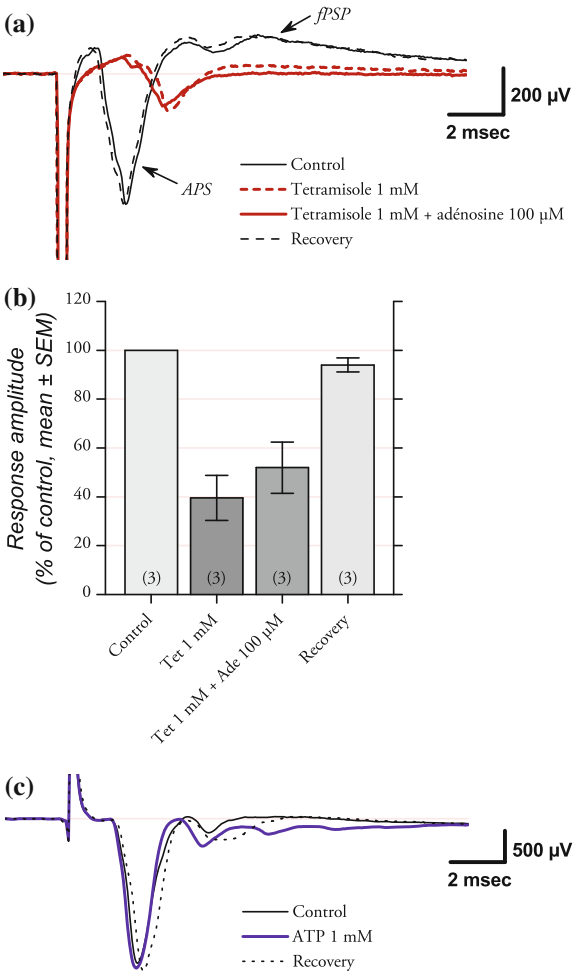


Fig. 12.6 High extracellular adenosine concentration does not compensate for the effect of tetramisole and the effect of tetramisole is not mimicked by high extracellular ATP concentration. **a** Comparison of the effects of tetramisole at 1 mM and tetramisole 1 mM + adenosine 100 µM. Recording in the supragranular layers of the somatosensory cortex, stimulation (100 µA, 300 µs) at the white matter-layer 6 border. Each trace is the average of 30 sweeps (1 min recording) in control ACSF (control and recovery), in ASCF + tetramisole 1 mM, and in ACSF + tetramisole 1 mM + adenosine 100 µm. As previously, tetramisole reduced the amplitude and increased the latency of the APS and eliminated the fPSP. Adenosine did not reverse the effect of tetramisole. **b** Bar graph summarizing the effect of tetramisole 1 mM or tetramisole 1 mM + adenosine (Ade) 100 µM on antidromic population spike amplitude. Amplitudes normalized by control values and expressed as a percentage of control response amplitude. Bar height: mean; error bars: SEM. **c** ATP (1 mM) does not mimic the effect of tetramisole. Recording in the supragranular layers of the somatosensory cortex, stimulation (100 µA, 300 µsec) in the white matter. Each trace is the average of 30 sweeps (1 min recording) in control ACSF (control and recovery) and in ASCF + ATP 1 mM

12.3.6 The Effect of Tetramisole Is not Stereo-Specific

Although they constitute two of the identified metabolites of TNAP, exogenously applied adenosine or PL did not compensate for the effect of tetramisole on neuronal response amplitude. In addition, the IC_{50} for neuronal response suppression by tetramisole (Fig. 12.2c, d) was much higher than that expected given the IC_{50} values reported for TNAP inhibition by levamisole or tetramisole in biochemical assays on cell extracts or cell cultures (Van Belle 1972, 1976a, 1976b; Goldstein et al. 1980; Anagnostou et al. 1996; Calhau et al. 2000; Picher et al. 2003; Sergienko and Millán 2010; Debray et al. 2013): the IC_{50} that has been reported in these studies is between 10 and 70 μM. In addition, the slope of the Hill equation fitted to our data was close to 3, while a Hill coefficient value close to 1 has been reported for the inhibition of TNAP by levamisole (Suzuki et al. 1994). Altogether, these negative results and discrepancies led us to suspect that tetramisole may have acted on a target *other than TNAP*.

Studies showed that levamisole (the levorotatory enantiomer of tetramisole), but not dexamisole (the dextrorotary enantiomer of tetramisole), is effective at inhibiting TNAP (Van Belle 1972, 1976b; Borgers 1973). If inhibition of TNAP activity was responsible for the effects we observed, then it should also be stereo-specific. It follows that the effect observed with a given concentration of tetramisole should be mimicked by levamisole at half that concentration. We therefore compared the effect of levamisole and tetramisole on antidromic response amplitude in 7 experiments. In each of these experiments 1 or 2 different concentrations of levamisole (0.5 or 1 mM) and identical concentrations of tetramisole were used. The prediction was that levamisole at 0.5 mM should have the same effect as tetramisole at 1 mM.

The results we obtained did not fit with this prediction. As illustrated in Fig. 12.7a, levamisole at 0.5 and 1 mM reduced both antidromic and orthodromic response amplitude and increased response latency, in a way comparable to tetramisole. Summary data in Fig. 12.7b show a highly significant reduction of antidromic response amplitude by both tetramisole and levamisole at 0.5 and 1 mM (ANOVA, $P < 0.0001$; PLSD Fisher test: $P < 0.0001$ for levamisole at 0.5 and 1 mM compared to control). However, the reduction of antidromic population spike amplitude by levamisole at 0.5 mM (67 ± 5 % of control response amplitude) was not significantly different ($P = 0.9$) from that of tetramisole at 0.5 mM (68 ± 3 % of control response amplitude). Likewise, the effect of levamisole at 1 mM did not differ significantly ($P = 0.3$) from that of tetramisole at 1 mM (46 ± 6 % and 40 ± 6 % of control response amplitude, respectively). On the other hand, levamisole at 0.5 mM was significantly less potent than tetramisole at 1 mM ($P < 0.0001$). These results show that the effect of tetramisole is not stereo-specific. This strongly suggests that the reduction of response amplitude induced by tetramisole was not mediated by TNAP.

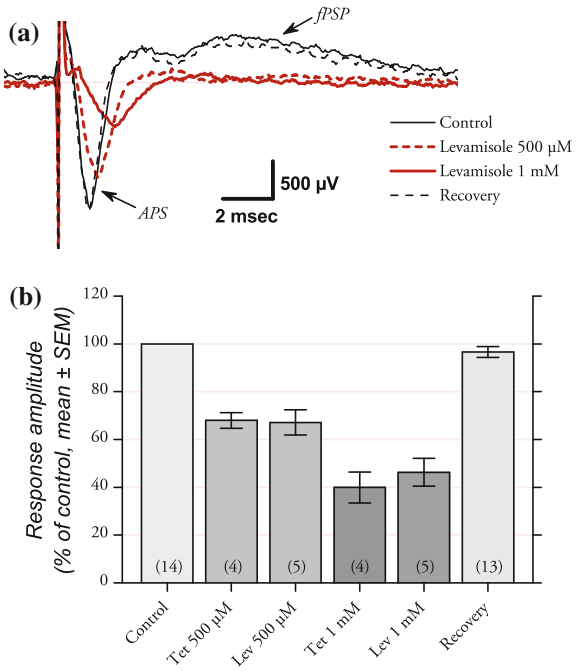


Fig. 12.7 Comparison of the effectiveness of tetramisole and levamisole on antidromic population spike amplitude reduction indicates lack of stereo-specificity in the action of tetramisole. **a** Example of the effects of levamisole at 0.5 and 1 mM. Recording in the supragranular layers of the barrel cortex, stimulation (65 µA, 200 µs) at the white matter-layer 6 border. Each trace is the average of 6 sweeps (1 min recording) in control ACSF (control and recovery) and in ASCF containing either 0.5 or 1 mM levamisole. As with tetramisole, levamisole reduced the amplitude and increased the latency of the antidromic population spike (APS). As with tetramisole, levamisole also suppressed the slow postsynaptic response (fPSP). **b** group data. "Tet" and "Lev" on x-axis refer to tetramisole and levamisole, respectively. Bar height represents the mean percent change in APS amplitude, error bars represent the SEM. Before averaging the amplitudes were normalized by the amplitude obtained in the control condition. The data presented here come from 7 experiments in which at least one concentration of levamisole and one concentration of tetramisole were tested in the same experiment. The total number of controls is 14 because control measurements were performed before both levamisole and tetramisole applications. One recovery not tested. Total number of measurements in levamisole and tetramisole sums to 18 because several concentrations of tetramisole or levamisole were eventually tested between one control and one recovery. The mean amplitude obtained in tetramisole 0.5 mM does not differ significantly from that obtained in levamisole 0.5 mM, and similarly when comparing tetramisole and levamisole at 1 mM

12.3.7 The Effect of Tetramisole Is not Mimicked by MLS-0038949, Another TNAP Inhibitor

An additional proof that tetramisole reduced neuronal response amplitude independently from TNAP inhibition was obtained by using another inhibitor of TNAP. This inhibitor—MLS-0038949—has been recently isolated and shows a very high

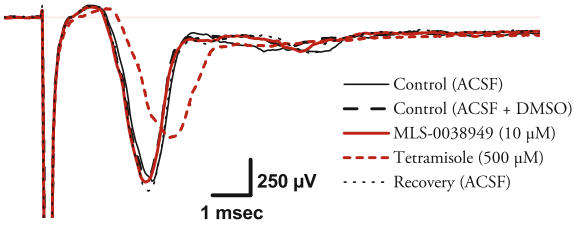


Fig. 12.8 Antidromic population spikes are not affected by MLS-0038949, another TNAP inhibitor. In this experiment, recording was performed in the supragranular layers of the barrel cortex and stimulation (100 µA, 200 µs) was applied in the white matter. Each trace is the average of 6 sweeps (1 min recording). Since MLS-0038949 was dissolved in DMSO, two controls were performed, one in regular ACSF and the other in ACSF + DMSO 0.1 %. DMSO had no effect the antidromic population spike. As in previous experiments, tetramisole (here at 0.5 mM) reduced the amplitude of the antidromic population spike and increased its latency. In contrast, MLS-0038949 (10 µM) had no effect on the amplitude and latency of the antidromic population spike

specificity for TNAP (Dahl et al. 2009). In Dahl et al. (2009) study, the IC_{50} for MLS-0038949 was about 0.2 µM and a complete inactivation of TNAP activity was achieved with 10 µM of MLS-0038949. In control histochemical experiments, we found that MLS-0038949 at 10 µM largely inhibited TNAP activity in mouse brain slices as assessed by histochemisty (NBT-BCIP method, as in Fonta and Imbert 2002) (not illustrated). We therefore examined the effect of MLS-0038949 at 10 µM in 3 experiments. Results of one of these experiments are presented in Fig. 12.8. Since MLS-0038949 was dissolved in DMSO to a final concentration of 0.1 % DMSO in ACSF (Methods), we first checked that DMSO 0.1 % per se had no effect on the response; the response obtained in ACSF + DMSO 0.1 % (Fig. 12.8, dashed line) is indistinguishable from that obtained in regular ACSF. We also checked that tetramisole had its usual action on the antidromic population spike; here again tetramisole (500 µM in this experiment) reduced the response amplitude and increased the peak response latency (Fig. 12.8, short dash). On the other hand, the antidromic population spikes was completely unaffected by MLS-0038949 at 10 µM (Fig. 12.8, thick solid line). The two other experiments failed to show any change on antidromic response amplitude and latency as well. These results confirm that the effects of tetramisole and levamisole on antidromic responses were not due to these compounds inhibiting TNAP activity.

12.3.8 Tetramisole Modifies Action Potential Shape and DV/Dt

In order to untangle how tetramisole reduced antidromic population spike amplitude, we next sought to examine its action on action potential electrogenesis using

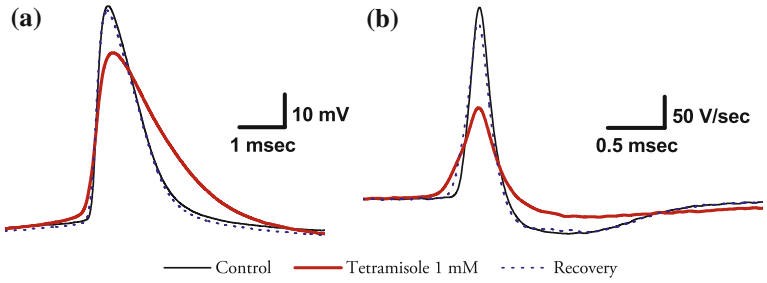


Fig. 12.9 Tetramisole prolongs action potential duration and reduces action potential rate-of-rise. **a** Action potentials were induced by intracellular current injection in an intracellularly recorded neuron (same cell as in Fig. 12.1h–j). Each trace corresponds to the average of 30 action potentials for each condition. Note reduced amplitude and prolonged duration in tetramisole 1 mM. **b** Each trace corresponds to the temporal derivative (dV/dt) of the action potential traces shown in **a**. The maximum rate of rise of the action potential (positive peak in the dV/dt) was reduced to 48 % of the control value in tetramisole 1 mM (control: 316 V/s; tetramisole 1 mM: 152 V/s; recovery: 302 V/s). These data suggest that the voltage-dependent sodium channels responsible for action potential upstroke were partially blocked by tetramisole 1 mM

intracellular recording in somatosensory cortex neurons. Although several cells have been recorded, only one has been held for long enough to obtain a control baseline, to examine the effect of tetramisole (1 mM), and to achieve a complete recovery. The results presented here are therefore very preliminary.

We observed that tetramisole (1 mM) modified the shape of the action potentials. This is illustrated in Fig. 12.9a, where each trace corresponds to the average of 30 action potential traces; these action potentials were induced by intracellular current injection; the mean firing rate induced by these current injections was around 40 spikes/sec in the three conditions. In comparison to the control trace, the action potential in the presence of 1 mM tetramisole showed a decrease in amplitude together with an increased duration. This effect of tetramisole was reversible.

We next computed the first derivative of the action potential (dV/dt). The positive peak in the dV/dt corresponds to the maximum rate-of-rise of the action potential. The maximum rate-of-rise, in turn, is a measure of the inward current underlying the rise of the action potential (Hodgkin and Katz 1949). This inward current is largely, if not entirely, determined by the voltage-dependent sodium conductance (Hodgkin and Katz 1949; Cohen and Strichartz 1977; Hondeghem 1978; Carter and Bean 2009). It can be seen in Fig. 12.9b that tetramisole (1 mM) reduced the maximum rate of rise of the action potential to half the control value. This effect was completely reversible. This result suggests that voltage-dependent sodium channels were partially blocked by tetramisole at 1 mM.

12.4 Discussion

Our study disclosed a new action of tetramisole and levamisole on neuronal signal transmission, which appears to be independent of these compounds acting on TNAP. We showed that tetramisole and levamisole reduced both antidromic and orthodromic response amplitude in mouse somatosensory cortex in a dose-dependent manner. This is a hitherto undocumented effect of tetramisole and levamisole. Yet our control experiments showed that these effects of tetramisole were not explained by inhibition of TNAP. Instead, the decrease of axonal conduction velocity and preliminary intracellular data suggest that tetramisole and levamisole may block voltage-dependent sodium channels. These results imply that, in addition to inhibiting TNAP, levamisole or tetramisole at concentration ≥1 or ≥2 mM completely inhibit all orthodromic and antidromic neuronal activity, respectively, and are therefore likely to suppress all processes that are activity-dependent in living brain cells, such as, among others, axonal growth, myelination or synaptic plasticity.

12.4.1 Suppression of Neuronal Activity by Tetramisole and Levamisole is not Due to TNAP Inhibition

Neuronal responses, both orthodromic and antidromic, were reduced by tetramisole and levamisole (Figs. 12.1, 12.2 and 12.7). These effects are unlikely to be due to TNAP inhibition, for several reasons:

First, the dose-response relationships (Fig. 12.2) appear to be very different from those reported for TNAP inhibition by tetramisole or levamisole. The IC_{50} we report here were 730 μM for antidromic response and 410 μM for orthodromic responses. Both values are much larger than those reported for TNAP inhibition by levamisole in biochemical assays on cell extracts or on cells cultures—in the range of 10–70 μM (Van Belle 1972, 1976a, b; Goldstein et al. 1980; Anagnostou et al. 1996; Picher et al. 2003; Sergienko and Millán 2010; Debray et al. 2013), including brain cell extracts (IC_{50} = 44 μM in Calhau et al. 2000). In addition the exponent of the Hill equation fitted to our data was close to 3, whereas TNAP inhibition has a Hill coefficient close to 1 (Suzuki et al. 1994).

Second, neuronal activity block was not rescued by the application of exogenous adenosine or PL (Figs. 12.5 and 12.6), although these were the metabolites whose extracellular concentrations were presumed to be reduced by TNAP inhibition in our experimental conditions (see Introduction).

Third, in our experiments equivalent concentrations of levamisole and tetramisole reduced neuronal responses by the same amount (Fig. 12.7). Given that tetramisole contains both levamisole and dexamisole in identical proportions, this implies that dexamisole had the same potency at inhibiting neuronal responses as levamisole. This further rules out TNAP inhibition as being responsible for the

effects reported here, given that only levamisole if effective at inhibiting TNAP (Van Belle 1972, 1976b; Borgers 1973).

Fourth and finally, blockage of neuronal activity by levamisole and tetramisole were not mimicked by MLS-0038949, a new and highly selective inhibitor of TNAP (Dahl et al. 2009) (Fig. 12.8).

Our results should not be interpreted as meaning that tetramisole or levamisole did not inhibit TNAP in our experimental conditions. Indeed, we performed control histochemical experiments (same method as Fonta and Imbert 2002) using brain slices prepared as for the electrophysiological experiments. These experiments confirmed that AP activity was effectively inhibited by tetramisole (not illustrated). Therefore, TNAP inhibition likely took place in our electrophysiological experiments but its consequences on synaptic transmission, if they occurred, were masked by the suppression of neuronal activity by tetramisole and levamisole.

12.4.2 Tetramisole and Levamisole May Suppress Neuronal Activity by Blocking Voltage-Dependent Sodium Channels

If TNAP inhibition was not responsible for the decrease in neuronal response amplitude produced by levamisole and tetramisole, then what other targets could explain the effects we observed? Two observations suggest that voltage-dependent sodium channels were blocked by tetramisole and levamisole.

First, with concentrations of tetramisole that did not completely suppress the responses, we observed that the latency of the antidromic population spikes increased (Fig. 12.3). The same effect was noticed when recording from axons in the corpus callosum (Fig. 12.4). These results indicate a slowing down of action potential propagation along the axons. Slowing down of action potential propagation speed in axons is not observed with compounds that block voltage-dependent potassium channels (Bostock et al. 1981; Fox and Ruan 1989), but it is typically observed with compounds that block voltage-dependent sodium channels such as TTX (Pinto et al. 2008), phenytoin (Le Quesne et al. 1976; Marcus et al. 1981) and several local anesthetics such as procaine (Franz and Perry 1974) and lidocaine (Raymond 1992; Yokota et al. 1994; De Col et al. 2008). Interestingly, a pharmacological study reported that levamisole possesses local anesthetic properties with a potency representing half that of lidocaine (Onuaguluchi and Igbo 1987); the ED_{50} obtained in this study was quite close to the IC_{50} we report here for the reduction of antidromic population spike amplitude.

Second, studies showed that the compounds that reduce axonal conduction velocity also reduce the peak height of the action potential dV/dt (TTX: Kao and Walker 1982; phenytoin: Selzer 1979, Hershkowitz and Ayala 1981; procaine: Ibusuki et al. 1998; lidocaine: Schwarz and Puil 1998). Since the peak height in the dV/dt is a measure of the sodium current underlying action potential electrogenesis,

the reduction of the dV/dt_{max} observed in the present study (Fig. 12.9) further supports the possibility that tetramisole blocks voltage-dependent sodium channels, although additional studies are required for a definitive confirmation. It is also presently unclear whether tetramisole directly blocks voltage-dependent sodium channels, or whether this effect is secondary to tetramisole acting on intracellular signaling pathways that control sodium channel gating properties.

Complete suppression of antidromic responses required a tetramisole concentration of about 2 mM whereas orthodromic responses were nearly completely suppressed with 1 mM tetramisole. Likewise, the IC_{50} for the two types of responses differed by a factor close to 2. We can only speculate on the origin of this discrepancy. The postsynaptic response is proportional to the amount of neurotransmitter released, which is determined by calcium influx inside the presynaptic terminals, which is itself the result of the activation of high threshold voltage-dependent calcium channels by sodium spikes in the axon terminals. Reduction of sodium spike amplitude may then results in a decreased neurotransmitter release but, to fit with our data, a nonlinearity in the presynaptic spike amplitude to postsynaptic response transform would be required. Alternatively, high threshold voltage-dependent calcium channels, which show some structural homologies with voltage-dependent sodium channels (e.g., Zakon 2012), may also be blocked by tetramisole.

12.4.3 Other Targets of Tetramisole and Levamisole in the Nervous System

It has long been shown that tetramisole and levamisole inhibit TNAP. The results we report here further suggest that tetramisole and levamisole may also block voltage-dependent sodium channels. In addition to these two targets, tetramisole and levamisole have been reported to have other actions in the nervous system. We shall first review studies that examined the effect of tetramisole and levamisole using electrophysiological approaches. These studies have been very few and concentrated mostly on nematode neuromuscular junction and mammalian peripheral nervous system.

12.4.3.1 Tetramisole, Levamisole and Acetylcholine Receptors

Tetramisole has originally been isolated for its anthelmintic properties (Thienpont et al. 1966; Raeymaekers et al. 1966). Initially based on screening tests in chicken, tetramisole was proven to be efficient against both intestinal and pulmonary nematode infections in a dozen mammalian species including human and tiger. Levamisole was shown to be the effective compound while dexamisole has no anthelmintic properties (Bullock et al. 1968). The action of tetramisole and

levamisole consists in a paralysis of the nematodes (Thienpont et al. 1966; Aceves et al. 1970; Atchison et al. 1992). Nematode paralysis results from a maintained depolarization of their muscle cells (Aceves et al. 1970; Harrow and Gration 1985; Atchison et al. 1992). Muscle cell depolarization is the consequence of levamisole acting as an agonist on neuromuscular acetylcholine (ACh) receptors of the nematode (Lewis et al. 1980; Harrow and Gration 1985; Robertson and Martin 1993).

Fortunately in mammals levamisole does not appear to be an agonist of the neuromuscular ACh receptor (Atchison et al. 1992; Rayes et al. 2004). Instead, on the basis of their pharmacological profiles (Lewis et al. 1980) and of genetic homologies (Fleming et al. 1997), it has been proposed that the neuromuscular ACh receptors that are activated by levamisole in the nematodes are homologous to ganglionic ACh receptors of mammals.

Nevertheless, the effect of levamisole on the ganglionic ACh receptors of mammals (α3β2 and α3β4 receptors) is unlike that observed in nematodes (Levandoski et al. 2003): levamisole applied alone has virtually no effect and therefore does not behave as an agonist of the ganglionic ACh receptors. On the other hand, levamisole modulates the response to exogenously applied ACh. Yet this modulation appears to be complex, as it depends on both levamisole and ACh concentrations. Thus the modulation exerted by levamisole can be either facilitatory or inhibitory.

Beside autonomic ganglia, the α3β2 and α3β4 receptors can be found in different brain regions but not in cortex (Perry et al. 2002). A large fraction of the nicotinic receptors found in cortex corresponds to the α4β2 subunits composition, for which, to our knowledge, levamisole action has not been examined. The α7* is another ACh receptor family that is largely represented in cortex (Paterson and Nordberg 2000) but levamisole does not seem to have any effect on this receptor family (Bartos et al. 2006).

12.4.3.2 Inhibition of Noradrenaline Reuptake by Dexamisole, Levamisole and Tetramisole in Peripheral Nervous System

In addition to cholinergic transmission, noradrenergic transmission in the peripheral nervous system also appears to be affected by tetramisole and levamisole (Vanhoutte et al. 1977; Pires et al. 1979; Gulati et al. 1985). In the various preparations studied (heart, smooth muscle, vas deferens), levamisole or tetramisole had no effect when applied alone and therefore do not appear to be agonists of noradrenaline (NA) receptors. Nonetheless, levamisole and tetramisole produced an enhancement of the response to endogenously released or to exogenously applied NA (Vanhoutte et al. 1977; Pires et al. 1979; Gulati et al. 1985). Response enhancement appeared to result from an inhibition of NA reuptake. The concentrations of levamisole or tetramisole required for near complete NA uptake inhibition were ≤40 μM. Dexamisole was more potent than levamisole (Vanhoutte et al. 1977).

The potency of levamisole represented half that of cocaine (Pires et al. 1979) and when NA uptake was already blocked by cocaine, levamisole had no further effect (Gulati et al. 1985), suggesting that levamisole and cocaine were affecting and competing for the same uptake mechanism. Several NA uptake inhibitors, such as cocaine and amphetamines, also inhibit dopamine uptake. Whether this is also the case for levamisole is not known to us but this could provide an explanation as to why illegal cocaine is now so often adulterated with levamisole.

In summary, tetramisole and levamisole may have—at least—4 different effects in the nervous system of mammals:

- Inhibition of TNAP, that may have multiple consequences resulting from interference with numerous PLP-dependent enzymes, including GABA synthesizing enzymes, or from modifications of the extracellular concentrations of ATP and adenosine.
- Modulation of responses mediated by ganglionic ACh receptors.
- Blockage of NA uptake mechanism.
- Blockage of voltage-dependent sodium channels, as reported in the present study.

In addition to these 4 targets, studies suggested that levamisole and tetramisole may affect additional targets in the nervous system, although these are less firmly established. Hence it has been proposed that tetramisole and levamisole could inhibit acetylcholinesterase (Eyre 1970) and monoamine oxidase (Vanhoutte et al. 1977). One study suggested that levamisole interferes with opiate receptors (Spector et al. 1998) but the levamisole doses that have been used in this study have been shown to be deadly in other studies (Mohammad et al. 2006; Rehni and Singh 2010).

12.4.4 Side Effects of Tetramisole and Levamisole Therapies

Levamisole and tetramisole received multiple medical applications: first as an anthelmintic, later on as an immunostimulant for the treatment of a number of diseases.

When used as an anthelmintic, levamisole and tetramisole are used in a single dose and, unless overdosing (Joly et al. 1998), this regimen has not been reported to have serious side effects.[3] On the other hand, serious side effects have been noticed with the chronic (several weeks or months) use of levamisole.

The chronic use of levamisole was largely based on studies reporting that tetramisole and levamisole possess immunostimulating properties (e.g., Renoux and Renoux 1972; Brugmans et al. 1973; Pabst and Crawford 1975; Spreafico et al. 1975;

[3]Adverse reactions after single levamisole or tetramisole doses have been consistently reported in veterinary medicine, and were usually attributed to their actions on ganglionic ACh receptors (reviewed in Hsu 1980).

Renoux et al. 1976; Hadden et al. 1977). Levamisole has thus been used to boost the immune system of patients suffering diseases that were supposed to be associated with, or that received treatments leading to, decreased immunity. Hence levamisole has been used for the treatment of rheumatoid arthritis (Schuermans 1975), of pediatric nephrotic syndrome (Tanphaichitr et al. 1980; Niaudet et al. 1984; Mongeau et al. 1988; British Association for Paediatric Nephrology 1991) and against various skin infections (reviewed in Hadden et al. 1977). Nevertheless, more recent studies have somehow questioned the efficacy of levamisole as an immunostimulant (e.g.: Toivanen et al. 1981; Webster et al. 1982; Aymard et al. 1984; Schiller et al. 1991; Ahmed et al. 1996). Likewise, studies questioned the efficacy of levamisole in the treatment of rheumatoid arthritis (Dinai and Pras 1975) or skin infections (Chang and Fiumara 1978; Seidlin and Straus 1984; Sanchez 2000).

Levamisole has also been used as an immunostimulant in cancer therapy. In this context, levamisole, when used alone, proved to be of limited efficacy (e.g., Toivanen et al. 1981; Treurniet-Donker et al. 1987; Arnaud et al. 1989; Barth and Morton 1995; Moertel et al. 1995) and has even been reported to be worse than placebo (e.g., Chlebowski et al. 1994). On the other hand, quite favorable outcomes have been reported when levamisole was combined with 5-fluorouracil in colon carcinoma treatment (e.g., Moertel et al. 1995).[4]

Thus, with few exceptions (colon carcinoma and pediatric nephrotic syndrome treatment), chronic levamisole treatments proved to be of limited efficacy. More problematical, it appeared that chronic levamisole treatment was associated with multiple side effects, some relatively mild and short-lived such as nausea and diarrhea, other much more severe and eventually life threatening such as dermatitis, cutaneous necrotizing vasculitis, leukopenia and agranulocytosis (e.g., Ruuskanen et al. 1976; Parkinson et al. 1977; MacFarlane and Bacon 1978; Scheinberg et al. 1978; Chang and Fiumara 1978; Toivanen et al. 1981; Niaudet et al. 1984; Moertel et al. 1990; reviewed in Symoens et al. 1978; Larocque and Hoffman 2012; Lee et al. 2012).

Chronic levamisole treatment also led to serious neurological side effects, in particular epileptic seizures and inflammatory leukoencephalopathy, that we shall discuss below.

Beforehand, we shall remind that, although levamisole and tetramisole have several putative targets in the CNS, the involvement of these targets in pathogenic processes depends on their sensitivity to levamisole, hence to the concentration of levamisole in the tissues. With respect to this issue, it is worth mentioning that the plasmatic levamisole concentration is <5 μM in most individuals receiving single, therapeutically relevant levamisole doses (Woestenborghs et al. 1981; Luyckx et al.

[4]In the context of cancer therapy, levamisole has also been reported to possess antiproliferative action in vitro (Kovach et al. 1992; Artwohl et al. 2000). However, the levamisole concentrations required to achieve significant effects in vitro appear to be much higher than the plasmatic concentration of levamisole in clinical therapy. Levamisole concentration in a range similar to the plasmatic concentration measured in patients fails to prevent the proliferation of cancer cell lines in vitro (Grem and Allegra 1989; Wiebke et al. 2003).

1983; Kouassi et al. 1986; Gwilt et al. 2000; Hess et al. 2014), although higher plasmatic concentration (up to 8 μM in Luyckx et al. 1983) have been observed in a minority of individuals. One study further suggested that levamisole may accumulate following repeated daily doses (Reid et al. 1998). In any event, plasmatic levamisole concentration was probably not much higher than 10−15 μM even in the most intensive cures. This rules out the new action of levamisole that we describe here since reduction of neuronal response amplitude and reduction of axonal conduction velocity required tetramisole concentration >100−200 μM. On the other hand, TNAP activity may be significantly affected by levamisole at around 10 μM.

12.4.4.1 Levamisole and Epileptic Seizures

Epileptic seizures have been observed, in all cases in children, during levamisole therapy for the treatment of pediatric nephrotic syndrome (Ruuskanen et al. 1976; Prieur et al. 1978; Palcoux et al. 1994). Seizures occurred after a delay of a few days or weeks after the beginning of the treatment. In Prieur et al. (1978) study, epileptic seizures were observed in 3/50 children. An action of levamisole on nicotinic receptors has been evoked (Palcoux et al. 1994) but alternative mechanisms are quite possible, in particular those involving TNAP inhibition by levamisole.

There are multiple types of epilepsy, each with its own etiology. Quite commonly, impairments of inhibitory mechanisms are involved. In this respect, it is well established that dysfunctions of both GABA- (reviewed in Cossart et al. 2005; Macdonald et al. 2010) and adenosine- (Pagonopoulou et al. 2006; Boison 2012) mediated signaling can lead to epilepsy. As outlined in Introduction, the synthesis of GABA involves TNAP through vitamin B6 metabolism, while the synthesis of extracellular adenosine partially depends, in some structures, on the ectonucleotidase activity of TNAP. That TNAP deficiencies result in epileptic seizures is well documented: in human, epileptic seizures are often observed in perinatal and infantile hypophosphatasia (e.g., Rathbun 1948; Fraser 1957; Béthenod et al. 1967; Baumgartner-Sigl et al. 2007; Balasubramaniam et al. 2010; Taketani et al. 2014; see also Chaps. 14 and 15) and TNAP KO mice, the murine model of severe hypophosphatasia, also present with epileptic seizures (Waymire et al. 1995; Narisawa et al. 1997). In parallel these mice show decreased level of GABA (Waymire et al. 1995; Fonta et al. 2012). In these mice, seizure incidence was reduced, though not definitely suppressed, by administration of PL (Waymire et al. 1995; Narisawa et al. 2001). Altogether, these studies point toward a PLP metabolism deficiency, and its consequence on, at least, GABA synthesis, as the origin of epileptic seizures in TNAP KO mice. In addition to reduced GABA level, we recently observed that adenosine concentration is reduced in the brain of 7 day-old $TNAP^{+/-}$ mice (Fonta et al. 2014). This suggests that reduced adenosine level may also participate in the generation of epileptic seizures in TNAP KO mice.

Since levamisole inhibits TNAP (among others), it is tempting do draw a parallel between the epileptogenic action of levamisole (Ruuskanen et al. 1976; Prieur et al. 1978; Palcoux et al. 1994) and the epileptic phenotype that has been observed in

both TNAP KO mice and in severe forms of hypophosphatasia. It is therefore possible that levamisole, by inhibiting TNAP, impairs the balance of excitation and inhibition by reducing the availability of GABA and/or adenosine, leading to epileptic seizures in susceptible individuals.[5]

12.4.4.2 Levamisole and Multifocal Inflammatory Leukoencephalopathy

The first cases of multifocal inflammatory leukoencephalopathy were reported in a small proportion (<1 %) of patients treated for colon carcinoma with 5-fluorouracil and levamisole (Hook et al. 1992; Kimmel and Schutt 1993; Leichman et al. 1993; Neu 1993; Bozik and Gilbert 1994; Critchley et al. 1994; Fassas et al. 1994; Ferroir et al. 1994; additional references in Wu et al. 2006). The patients presented neurological disorders such as diplopia, dysarthria, paresthesia, ataxia, short-term memory impairments and/or loss of consciousness. In all cases MRI examination revealed leukoencephalopathy with multiple white matter lesions. Histological examinations performed from biopsies typically revealed extensive myelin loss and gliosis, but no or only marginal axonal loss. Perivascular lymphocytes and macrophage infiltration were consistently observed, demonstrating an inflammatory process in the leukoencephalopathy (Hook et al. 1992; Neu 1993; Fassas et al. 1994; Bozik and Gilbert 1994; Chen et al. 1994; Savarese et al. 1996; Murray et al. 1997; Israel et al. 2000; Liu et al. 2006; Wu et al. 2006). It was initially unclear whether the lesions were due to levamisole or to 5-fluorouracil but further studies and case reports indicated that levamisole alone could be responsible (Chen et al. 1994; Kimmel et al. 1995; Lucia et al. 1996; Liu et al. 2006; Wu et al. 2006). Years earlier, an experimental study performed in dogs had revealed that chronic levamisole intake resulted in mononuclear cell infiltration in the brain parenchyma, eventually accompanied by demyelination, in the vast majority of tested animals (Vandevelde et al. 1978).

The clinical features of the inflammatory multifocal leukoencephalopathy induced by levamisole are reminiscent of those observed in multiple sclerosis. This is further supported by studies reporting interaction between multiple sclerosis and levamisole: in a clinical trial, levamisole was used with the purpose to alleviate the symptoms of multiple sclerosis, yet levamisole had the opposite effect: it worsened the clinical picture in 5 of the 7 patients (Dau et al. 1976); this led to the premature interruption of the trial. In studies using rat or mouse models of multiple sclerosis (experimental allergic encephalomyelitis, EAE), levamisole increased both the incidence and the severity of the disease, and inflammation and demyelination were exacerbated in animals receiving levamisole in comparison to non-treated animals (Spreafico et al. 1975; Hertz and Deghenghi 1985; Lucchinetti et al. 1997).

[5]The epileptic seizures that have been observed in the course of chronic levamisole treatments have all been reported in children but not in adults. This offers another interesting parallel with vitamin B6 metabolism alteration: experimental studies showed that vitamin B6 deficiency results in epileptic crisis in young animals but not in the adults (Daniel et al. 1942; Guilarte 1989).

It is generally admitted that the etiology of multiple sclerosis involves an autoimmune component (for review: Goverman 2009; Cieślak et al. 2011; Stys 2013). In particular, T cells are suspected to play a prominent role in multiple sclerosis. In parallel, it has been reported that the increase in cell-mediated immunity by levamisole largely rests on the recruitment and activation of T cells (e.g., Renoux et al. 1976). Thus the general belief was that levamisole-induced multifocal leukoencephalopathy was the consequence of the immunostimulating action of levamisole in patients with subclinical multiple sclerosis.

Yet, to our knowledge none of the studies that examined the etiology of levamisole-induced multifocal leukoencephalopathy considered the possibility that inhibition of TNAP by levamisole could be involved. A support for this possibility is that TNAP is strategically located on Ranvier's node (Pinner et al. 1964; Mori and Nagano 1985; Fonta et al. 2005; Hanics et al. 2012). Here we propose two, non exclusive hypothetical mechanisms, that both involve TNAP inhibition in levamisole-induced multifocal leukoencephalopathy.

The first mechanism concerns the inflammatory reaction observed in levamisole-induced leukoencephalopathy and a possible role for TNAP in this process through its ectonucleotidase activity. There is now ample evidence that inflammation is tightly associated with purinergic signaling (reviewed in: Khakh and North 2006; Di Virgilio et al. 2009; Trautmann 2009; Cieślak et al. 2011; Sperlágh and Illes 2014). To make a long story short, it has been shown that rises in the extracellular concentration of ATP is pro-inflammatory, whereas extracellular adenosine acts as an anti-inflammatory. It is also worth mentioning that purinergic signaling is likely to control the severity of the deficits and the extent of the lesions in EAE: the inactivation of both adenosine A1 and $A2_A$ receptors aggravates inflammation and demyelination (Tsutsui et al. 2004; Yao et al. 2012) while blockade or inactivation of ATP $P2X_7$ receptors has the opposite effect (Matute et al. 2007; Sharp et al. 2008).

ATP is released by axons, in proportion to action potential firing rate (reviewed in Fields, 2011). In the extracellular space, ATP is rapidly degraded by ectonucleotidases and TNAP, through its strategic location on Ranvier's node, may play an active role in setting the ATP/adenosine balance at this location. Inhibition of TNAP by levamisole may thus lead to an increased extracellular ATP concentration and/or a decreased extracellular adenosine concentration in the close vicinity of myelinated structures. This could trigger a series of reactions, in particular secretion of a variety of pro-inflammatory cytokines, that would ultimately result in monocyte infiltration and myelin loss. This does not rule out the involvement of an autoimmune component in levamisole-induced leukoencephalopathy. If this was the case, levamisole would rather amplify the autoimmune response. In this sense TNAP inhibition by levamisole could be considered as "immunostimulating". However this immunostimulation would not be due to levamisole acting directly on immune cells (Schiller et al. 1991); instead, by altering the extracellular ATP/adenosine balance, levamisole may have an action upstream from the immune system.

The second mechanism where TNAP could play a role concerns the remyelination that may be impaired in levamisole-induced leukoencephalopathy. TNAP is

likely to intervene in myelin synthesis. This is supported by the observation that TNAP is highly expressed on axons during development, that is, during myelination (Fonta and Imbert 2002; Fonta et al. 2005) and by the fact that myelination is altered in TNAP KO mice (Hanics et al. 2012). The way TNAP participates in myelination may again involve its function as an ectonucleotidase.

Studies showed that myelination is in large part promoted by action potential firing in the axons (Demerens et al. 1996). Spike firing in the axons leads to ATP release (Fields 2011). Early stages of myelination, which consist in reducing oligodendrocyte precursor cells proliferation and in their differentiation in mature oligodendrocytes, requires adenosine signaling (Stevens et al. 2002). On the other hand, myelination by mature oligodendrocyte is promoted by extracellular ATP, which induces the release of a cytokine, leukemia inhibitory factor, by astrocytes (Ishibashi et al. 2006). Yet leukemia inhibitory factor has a dual action: it promotes myelination at low concentration but inhibits it when present at high concentration. Here again, inhibition of TNAP by levamisole may alter the extracellular ATP/adenosine balance, which would result in compromising (re)myelination: first, TNAP inhibition by levamisole may reduce the availability of adenosine required in the early stages of myelination; secondly, by reducing ATP degradation, TNAP inhibition may lead to an excess of leukemia inhibitory factor in the later stages of myelination.[6]

12.4.4.3 Levamisole and Tetramisole as Cocaine Adulterants

Between the sixties and the nineties, tetramisole and levamisole have been used in a considerable number of medical treatments. Yet, as briefly summarized above, tetramisole and levamisole appeared to be of limited efficacy in most of their applications and, more problematically, their chronic use resulted in serious side-effects in a non-negligible proportion of the patients. For these reasons, tetramisole and levamisole have been discontinued in most countries at the turn of the century, or their uses have been drastically restricted.[7]

[6]In addition to its involvement as an ectonucleotidase, TNAP could also intervene in myelination through vitamin B6 metabolism. In support for a role of PLP in myelination, it has been reported that vitamin B6 deficiency during development results in altered myelination (Lott et al. 1978; Kirksey et al. 1990). Indeed, PLP is a cofactor for two enzymes (serine palmitoyltransferase and sphingosine-1-phosphatelyase) involved in sphingolipid synthesis (Bourquin et al. 2011). Among sphingolipids, sphingomyelin, as expected from its name, happens to be particularly abundant in myelin. On the other hand, if myelination impairments observed in levamisole-induced multifocal leukoencephalopathy were consecutive to altered vitamin B6 metabolism, another PLP-dependent process, GABA synthesis, should also be impaired. This should result in occurrences of epileptic seizures in patients with levamisole-induced leukoencephalopathy, which, to our knowledge, have not been reported in case studies.

[7]In France for example, levamisole can still be used for the treatment of pediatric nephrotic syndrome, but under very restrictive conditions.

Yet, near the middle of the last decade, levamisole and tetramisole made an unexpected come-back as it turns out that they are now used as illegal cocaine adulterants (Morley et al. 2006; Fucci 2007; LeGatt 2009). Sixty to eighty percent of the cocaine seized around 2010 in North America or in Europe appeared to contain levamisole (Schneider and Meys 2011; Casale et al. 2012; Lahaie 2012), or, more rarely, tetramisole (Casale et al. 2012). The reasons why traffickers adopted such adulterants have not been trumpeted far and wide. One possibility is that levamisole may be degraded to aminorex, an amphetamine-like compound. Aminorex has been detected in horses that were treated with levamisole (see Larocque and Hoffman 2012) but aminorex is barely detectable in human after levamisole intake (Hess et al. 2014). A more likely reason is that, as inhibitors of noradrenaline uptake, levamisole and dexamisole may have cocaine-like action (see above).

As a result, the undesirable consequences that formerly were observed during chronic levamisole treatment are now observed in a number of cocaine users (reviewed in: Buchanan and Lavonas 2012; Larocque and Hoffman 2012; Lee et al. 2012). This includes thrombocytopenia, agranulocytosis and leukopenia, dermatitis and vasculitis. Occurrence of inflammatory multifocal leukoencephalopathy has also been reported in illegal cocaine users; although not definitively demonstrated, levamisole was suspected to be responsible for these rare instances of leukoencephalopathy (Gilbert 2011; Blanc et al. 2012; González-Duarte and Williams 2013).

Levamisole and tetramisole side-effects, including those that affect the nervous system, might not be something of the past. Understanding the mechanisms responsible for these side-effects may become crucially important in the near future. As we hypothesized above, TNAP may be a key player in these mechanisms, which certainly deserve further study.

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References

Abbracchio MP, Burnstock G, Verkhratsky A, Zimmermann H (2009) Purinergic signalling in the nervous system: an overview. Trends Neurosci 32:19–29

Aceves J, Erlij D, Martínez-Marañón R (1970) The mechanism of the paralysing action of tetramisole on Ascaris somatic muscle. Br J Pharmacol 38:602–607

Ahmed FY, Leonard GA, A'Hern R, Taylor AE, Lorentzos A, Atkinson H, Moore J, Nicolson MC, Riches PG, Gore ME (1996) A randomised dose escalation study of subcutaneous interleukin 2 with and without levamisole in patients with metastatic renal cell carcinoma or malignant melanoma. Br J Cancer 74:1109–1113

Anagnostou F, Plas C, Forest N (1996) Ecto-alkaline phosphatase considered as levamisole-sensitive phosphohydrolase at physiological pH range during mineralization in cultured fetal calvaria cells. J Cell Biochem 60:484–494

Arnaud JP, Buyse M, Nordlinger B, Martin F, Pector JC, Zeitoun P, Adloff A, Duez N (1989) Adjuvant therapy of poor prognosis colon cancer with levamisole: results of an EORTC double-blind randomized clinical trial. Br J Surg 76:284–289

Artwohl M, Hölzenbein T, Wagner L, Freudenthaler A, Waldhäusl W, Baumgartner-Parzer SM (2000) Levamisole induced apoptosis in cultured vascular endothelial cells. Br J Pharmacol 131:1577–1583

Atchison WD, Geary TG, Manning B, VandeWaa EA, Thompson DP (1992) Comparative neuromuscular blocking actions of levamisole and pyrantel-type anthelmintics on rat and gastrointestinal nematode somatic muscle. Toxicol Appl Pharmacol 112:133–143

Aymard JP, Janot C, Thibaut G, Bertrand F, Legras B, Lederlin P, Streiff F (1984) Levamisole in chronic lymphocytic leukaemia: a prospective study of 15 patients. Acta Haematol 71:316–321

Balasubramaniam S, Bowling F, Carpenter K, Earl J, Chaitow J, Pitt J, Mornet E, Sillence D, Ellaway C (2010) Perinatal hypophosphatasia presenting as neonatal epileptic encephalopathy with abnormal neurotransmitter metabolism secondary to reduced co-factor pyridoxal-5′-phosphate availability. J Inherit Metab Dis Suppl 3:S25–S33

Barth A, Morton DL (1995) The role of adjuvant therapy in melanoma management. Cancer 75 (S2):726–734

Bartos M, Rayes D, Bouzat C (2006) Molecular determinants of pyrantel selectivity in nicotinic receptors. Mol Pharmacol 70:1307–1318

Baumgartner-Sigl S, Haberlandt E, Mumm S, Scholl-Bürgi S, Sergi C, Ryan L, Ericson KL, Whyte MP, Högler W (2007) Pyridoxine-responsive seizures as the first symptom of infantile hypophosphatasia caused by two novel missense mutations (c.677T > C, p. M226T; c.1112C > T, p.T371I) of the tissue-nonspecific alkaline phosphatase gene. Bone 40:1655–1661

Béthenod M, Cotte MF, Collombel C, Fréderich A, Cotte J (1967) Hypophosphatasie à révélation néo-natale. Amélioration osseuse. Encéphalopathie convulsivante fatale. Ann Pediatr (Paris) 14:835–841

Blanc PD, Chin C, Lynch KL (2012) Multifocal inflammatory leukoencephalopathy associated with cocaine abuse: is levamisole responsible? Clin Toxicol 50:534–535

Boison D (2012) Adenosine dysfunction in epilepsy. Glia 60:1234–1243

Borgers M (1973) The cytochemical application of new potent inhibitors of alkaline phosphatases. J Histochem Cytochem 21:812–824

Bostock H, Sears TA, Sherratt RM (1981) The effects of 4-aminopyryridine and tetraethylammonium ions on normal and demyelinated mammalian nerve fibres. J Physiol 313:301–315

Bourne G (1943) The distribution of alkaline phosphatase in various tissues. Quart J exp physiol 32:1–20

Bourquin F, Capitani G, Grütter MG (2011) PLP-dependent enzymes as entry and exit gates of sphingolipid metabolism. Protein Sci 20:1492–1508

Bozik ME, Gilbert MR (1994) The role of levamisole in 5-fluorouracil-levamisole-associated multifocal leukoencephalopathy. Ann Neurol 36:295

British Association for Paediatric Nephrology (1991) Levamisole for corticosteroid-dependent nephrotic syndrome in childhood. Lancet 337: 1555–1557

Brugmans J, Schuermans V, De Cock W, Thienpont D, Janssen P, Verhaegen H, Van Nimmen L, Louwagie AC, Stevens E (1973) Restoration of host defense mechanisms in man by levamisole. Life Sci 13:1499–1504

Brumberg JC, Nowak LG, McCormick DA (2000) Ionic mechanisms underlying repetitive high-frequency burst firing in supragranular cortical neurons. J Neurosci 20:4829–4843

Brun-Heath I, Ermonval M, Chabrol E, Xiao J, Palkovits M, Lyck R, Miller F, Couraud PO, Mornet E, Fonta C (2011) Differential expression of the bone and the liver tissue non-specific alkaline phosphatase isoforms in brain tissues. Cell Tissue Res 343:521–536

Buchanan JA, Lavonas EJ (2012) Agranulocytosis and other consequences due to use of illicit cocaine contaminated with levamisole. Curr Opin Hematol 19:27–31

Bullock MW, Hand JJ, Waletzky E (1968) Resolution and racemization of dl-tetramisole, dl-6-phenyl-2,3,5,6-tetrahydroimidazo-[2,1-b]thiazole. J Med Chem 11:169–171

Butt AM (2011) ATP: a ubiquitous gliotransmitter integrating neuron-glial networks. Semin Cell Dev Biol 22:205–213

Calhau C, Martel F, Hipólito-Reis C, Azevedo I (2000) Differences between duodenal and jejunal rat alkaline phosphatase. Clin Biochem 33:571–577

Carter BC, Bean BP (2009) Sodium entry during action potentials of mammalian neurons: incomplete inactivation and reduced metabolic efficiency in fast-spiking neurons. Neuron 64:898–909

Casale JF, Colley VL, Legatt DF (2012) Determination of phenyltetrahydroimidazothiazole enantiomers (Levamisole/Dexamisole) in illicit cocaine seizures and in the urine of cocaine abusers via chiral capillary gas chromatography-flame-ionization detection: clinical and forensic perspectives. J Anal Toxicol 36:130–135

Chang TW, Fiumara N (1978) Treatment with levamisole of recurrent herpes genitalis. Antimicrob Agents Chemother 13:809–812

Chen TC, Hinton DR, Leichman L, Atkinson RD, Apuzzo ML, Couldwell WT (1994) Multifocal inflammatory leukoencephalopathy associated with levamisole and 5-fluorouracil: case report. Neurosurgery 35:1138–1142

Chlebowski RTL, Lillington L, Nystrom JS, Sayre J (1994) Late mortality and levamisole adjuvant therapy in colorectal cancer. Br J Cancer 69:1094–1097

Ciancaglini P, Yadav MC, Simão AM, Narisawa S, Pizauro JM, Farquharson C, Hoylaerts MF, Millán JL (2010) Kinetic analysis of substrate utilization by native and TNAP-, NPP1-, or PHOSPHO1-deficient matrix vesicles. J Bone Miner Res 25:716–723

Cieślak M, Kukulski F, Komoszyński M (2011) Emerging role of extracellular nucleotides and adenosine in multiple sclerosis. Purinergic Signal 7:393–402

Cohen IS, Strichartz GR (1977) On the voltage-dependent action of tetrodotoxin. Biophys J 17:275–279

Collins GG, Anson J (1985) Adenosine A1 receptors mediate the inhibitory effects of exogenous adenosine in the rat olfactory cortex slice. Neuropharmacology 24:1077–1084

Connors BW, Malenka RC, Silva LR (1988) Two inhibitory postsynaptic potentials, and $GABA_A$ and $GABA_B$ receptor-mediated responses in neocortex of rat and cat. J Physiol 406:443–468

Cossart R, Bernard C, Ben-Ari Y (2005) Multiple facets of GABAergic neurons and synapses: multiple fates of GABA signalling in epilepsies. Trends Neurosci 28:108–115

Critchley P, Abbott R, Madden FJ (1994) Multifocal inflammatory leukoencephalopathy developing in a patient receiving 5-fluorouracil and levamisole. Clin Oncol (R Coll Radiol) 6:406

Dahl R, Sergienko EA, Su Y, Mostofi YS, Yang L, Simao AM, Narisawa S, Brown B, Mangravita-Novo A, Vicchiarelli M, Smith LH, O'Neill WC, Millán JL, Cosford ND (2009) Discovery and validation of a series of aryl sulfonamides as selective inhibitors of tissue-nonspecific alkaline phosphatase (TNAP). J Med Chem 52:6919–6925

Daniel EP, Kline OD, Tolle CD (1942) A convulsive syndrome in young rats associated with pyridoxine deficiency. J Nutr 23:205–216

Dau PC, Johnson KP, Spitler LE (1976) The effect of levamisole on cellular immunity in multiple sclerosis. Clin Exp Immunol 26:302–309

de Bernard B, Bianco P, Bonucci E, Costantini M, Lunazzi GC, Martinuzzi P, Modricky C, Moro L, Panfili E, Pollesello P, Stagni N, Vittur F (1986) Biochemical and immunohistochemical evidence that in cartilage an alkaline phosphatase is a Ca^{2+}-binding glycoprotein. J Cell Biol 103:1615–1623

De Col R, Messlinger K, Carr RW (2008) Conduction velocity is regulated by sodium channel inactivation in unmyelinated axons innervating the rat cranial meninges. J Physiol 586:1089–1103

Debray J, Chang L, Marquès S, Pellet-Rostaing S, Le Duy D, Mebarek S, Buchet R, Magne D, Popowycz F, Lemaire M (2013) Inhibitors of tissue-nonspecific alkaline phosphatase: design, synthesis, kinetics, biomineralization and cellular tests. Bioorg Med Chem 21:7981–7987

Deisz RA, Prince DA (1989) Frequency-dependent depression of inhibition in guinea-pig neocortex in vitro by $GABA_B$ receptor feed-back on GABA release. J Physiol 412:513–541

Demerens C, Stankoff B, Logak M, Anglade P, Allinquant B, Couraud F, Zalc B, Lubetzki C (1996) Induction of myelination in the central nervous system by electrical activity. Proc Natl Acad Sci USA 93:9887–9892

Di Virgilio F, Ceruti S, Bramanti P, Abbracchio MP (2009) Purinergic signalling in inflammation of the central nervous system. Trends Neurosci 32:79–87

Dinai Y, Pras M (1975) Levamisole in rheumatoid arthritis. Lancet 2(7934):556

Ermonval M, Baudry A, Baychelier F, Pradines E, Pietri M, Oda K, Schneider B, Mouillet-Richard S, Launay JM, Kellermann O (2009) The cellular prion protein interacts with the tissue non-specific alkaline phosphatase in membrane microdomains of bioaminergic neuronal cells. PLoS ONE 4:e6497

Eyre P (1970) Some pharmacodynamic effects of the nematocides: methyridine, tetramisole and pyrantel. J Pharm Pharmacol 22:26–36

Fassas AB, Gattani AM, Morgello S (1994) Cerebral demyelination with 5-fluorouracil and levamisole. Cancer Invest 12:379–383

Ferroir JP, Fenelon G, Beaugerie L, Avenin Recoing D (1994) Leucoencéphalopathie multifocale inflammatoire, complication de la chimiothérapie par 5 fluorouracil et levamisole. Rev Neurol (Paris) 150:471–474

Fields RD, Burnstock G (2006) Purinergic signalling in neuron-glia interactions. Nat Rev Neurosci 7:423–436

Fields RD (2011) Nonsynaptic and nonvesicular ATP release from neurons and relevance to neuron-glia signaling. Semin Cell Dev Biol 22:214–219

Fleming JT, Squire MD, Barnes TM, Tornoe C, Matsuda K, Ahnn J, Fire A, Sulston JE, Barnard EA, Sattelle DB, Lewis JA (1997) Caenorhabditis elegans levamisole resistance genes lev-1, unc-29, and unc-38 encode functional nicotinic acetylcholine receptor subunits. J Neurosci 17:5843–5857

Fonta C, Imbert M (2002) Vascularization in the primate visual cortex during development. Cereb Cortex 12:199–211

Fonta C, Négyessy L, Renaud L, Barone P (2004) Areal and subcellular localization of the ubiquitous alkaline phosphatase in the primate cerebral cortex: evidence for a role in neurotransmission. Cereb Cortex 14:595–609

Fonta C, Negyessy L, Renaud L, Barone P (2005) Postnatal development of alkaline phosphatase activity correlates with the maturation of neurotransmission in the cerebral cortex. J Comp Neurol 486:179–196

Fonta C, Négyessy L, Brun-Heath I, Ermonval M, Czege D, Nowak LG, Frances B, Xiao JS, Mornet E, Millán JL (2012) Bone TNAP in the brain: functions in neurotransmission. Bull Group Int Rech Sci Stomatol Odontol 51:27

Fonta C, Cruz T, Rosay B, Millán JL, Balayssac S, Gilard V, Malet-Martino M, Nowak LG (2014) A dual approach to explore the functions of the tissue nonspecific alkaline phosphatase (TNAP) in the brain. Ninth FENS Forum of Neuroscience, Milan, Italy. Abstract Number: FENS-1232

Fontanez DE, Porter JT (2006) Adenosine A1 receptors decrease thalamic excitation of inhibitory and excitatory neurons in the barrel cortex. Neuroscience 137:1177–1184

Fox DA, Ruan DY (1989) Time- and frequency-dependent effects of potassium channel blockers on large and medium diameter optic tract axons. Brain Res 498:229–242

Franz DN, Perry RS (1974) Mechanisms for differential block among single myelinated and non-myelinated axons by procaine. J Physiol 236:193–210

Fraser D (1957) Hypophosphatasia. Am J Med 22:730–746

Friede RL (1966) A quantitative mapping of alkaline phosphatase in the brain of the rhesus monkey. J Neurochem 13:197–203

Fucci N (2007) Unusual adulterants in cocaine seized on Italian clandestine market. Forensic Sci Int 172:e1

Gilbert JW (2011) J Neuroimaging 21:e188

Goldstein DJ, Rogers CE, Harris H (1980) Expression of alkaline phosphatase loci in mammalian tissues. Proc Natl Acad Sci USA 77:2857–2860

González-Duarte A, Williams R (2013) Cocaine-induced recurrent leukoencephalopathy. Neuroradiol J 26:511–513

Goverman J (2009) Autoimmune T cell responses in the central nervous system. Nat Rev Immunol 9:393–407

Grem JL, Allegra CJ (1989) Toxicity of levamisole and 5-fluorouracil in human colon carcinoma cells. J Natl Cancer Inst 81:1413–1417

Guilarte TR (1989) Regional changes in the concentrations of glutamate, glycine, taurine, and GABA in the vitamin B-6 deficient developing rat brain: association with neonatal seizures. Neurochem Res 14:889–897

Gulati OD, Hemavathi KG, Joshi DP (1985) Interactions of levamisole with some autonomic drugs on guinea-pig vas deferens. J Auton Pharmacol 5:19–23

Gwilt P, Tempero M, Kremer A, Connolly M, Ding C (2000) Pharmacokinetics of levamisole in cancer patients treated with 5-fluorouracil. Cancer Chemother Pharmacol 45:247–251

Hadden JW, Lopez C, O'Reilly RJ, Hadden EM (1977) Levamisole and inosiplex: antiviral agents with immunopotentiating action. Ann NY Acad Sci 284:139–152

Hanics J, Barna J, Xiao J, Millán JL, Fonta C, Négyessy L (2012) Ablation of TNAP function compromises myelination and synaptogenesis in the mouse brain. Cell Tissue Res 349:459–471

Harrow ID, Gration KAF (1985) Mode of action of the anthelmintics morantel, pyrantel and levamisole on muscle cell membrane of the nematode Ascaris suum. Pestic Sci 16:662–672

Hershkowitz N, Ayala GF (1981) Effects of phenytoin on pyramidal neurons of the rat hippocampus. Brain Res 208:487–492

Hertz F, Deghenghi R (1985) Effect of rat and beta-human interferons on hyperacute experimental allergic encephalomyelitis in rats. Agents Actions 16:397–403

Hess C, Ritke N, Sydow K, Mehling LM, Ruehs H, Madea B, Musshoff F (2014) Determination of levamisole, aminorex, and pemoline in plasma by means of liquid chromatography-mass spectrometry and application to a pharmacokinetic study of levamisole. Drug Test Anal 6:1049–1054

Hodgkin AL, Katz B (1949) The effect of sodium ions on the electrical activity of giant axon of the squid. J Physiol 108:37–77

Hondeghem LM (1978) Validity of V_{max} as a measure of the sodium current in cardiac and nervous tissues. Biophys J 23:147–152

Hook CC, Kimmel DW, Kvols LK, Scheithauer BW, Forsyth PA, Rubin J, Moertel CG, Rodriguez M (1992) Multifocal inflammatory leukoencephalopathy with 5-fluorouracil and levamisole. Ann Neurol 3:262–267

Hoshi K, Amizuka N, Oda K, Ikehara Y, Ozawa H (1997) Immunolocalization of tissue non-specific alkaline phosphatase in mice. Histochem Cell Biol 107:183–191

Howe JR, Sutor B, Zieglgänsberger W (1987a) Characteristics of long-duration inhibitory postsynaptic potentials in rat neocortical neurons in vitro. Cell Mol Neurobiol 7:1–18

Howe JR, Sutor B, Zieglgänsberger W (1987b) Baclofen reduces postsynaptic potentials of rat cortical neurones by an action other than its hyperpolarizing action. J Physiol 384:539–569

Hsu WH (1980) Toxicity and drug interactions of levamisole. J Am Vet Med Assoc 176:1166–1169

Ibusuki S, Katsuki H, Takasaki M (1998) The effects of extracellular pH with and without bicarbonate on intracellular procaine concentrations and anesthetic effects in crayfish giant axons. Anesthesiology 88:1549–1557

Irnich D, Tracey DJ, Polten J, Burgstahler R, Grafe P (2002) ATP stimulates peripheral axons in human, rat and mouse—differential involvement of A(2B) adenosine and P2X purinergic receptors. Neuroscience 110:123–129

Ishibashi T, Dakin KA, Stevens B, Lee PR, Kozlov SV, Stewart CL, Fields RD (2006) Astrocytes promote myelination in response to electrical impulses. Neuron 49:823–832

Israel ZH, Lossos A, Barak V, Soffer D, Siegal T (2000) Multifocal demyelinative leukoencephalopathy associated with 5-fluorouracil and levamisole. Acta Oncol 39:117–120

Joly C, Palisse M, Ribbe D, De Calmes O, Genevey P (1998) Intoxication aiguë au levamisole "à dose de cheval". La revue des SAMU 1998:237–240

Kao CY, Walker SE (1982) Active groups of saxitoxin and tetrodotoxin as deduced from actions of saxitoxin analogues on frog muscle and squid axon. J Physiol 323:619–637

Khakh BS, North RA (2006) P2X receptors as cell-surface ATP sensors in health and disease. Nature 442:527–532

Kimmel DW, Schutt AJ (1993) Multifocal leukoencephalopathy: occurrence during 5-fluorouracil and levamisole therapy and resolution after discontinuation of chemotherapy. Mayo Clin Proc 68:363–365

Kimmel DW, Wijdicks EF, Rodriguez M (1995) Multifocal inflammatory leukoencephalopathy associated with levamisole therapy. Neurology 45:374–376

Kirksey A, Morré DM, Wasynczuk AZ (1990) Neuronal development in vitamin B6 deficiency. Ann NY Acad Sci 585:202–218

Kouassi E, Caillé G, Léry L, Larivière L, Vézina M (1986) Novel assay and pharmacokinetics of levamisole and p-hydroxylevamisole in human plasma and urine. Biopharm Drug Dispos 7:71–89

Kovach JS, Svingen PA, Schaid DJ (1992) Levamisole potentiation of fluorouracil antiproliferative activity mimicked by orthovanadate, an inhibitor of tyrosine phosphatase. J Natl Cancer Inst 84:515–519

Lahaie E (2012) Quel est le produit qui circule? In: Pousset M (ed) Observatoire Français des Drogues et des Toxicomanes; cocaïne, données essentielles, pp 35–41

Landow H, Kabat EA, Newman W (1942) Distribution of alkaline phosphatase in normal and in neoplastic tissues of the nervous system. A histochemical study. Arch Neurol Psychiatry 48:518–530

Langer D, Hammer K, Koszalka P, Schrader J, Robson S, Zimmermann H (2008) Distribution of ectonucleotidases in the rodent brain revisited. Cell Tissue Res 334:199–217

Larocque A, Hoffman RS (2012) Levamisole in cocaine: unexpected news from an old acquaintance. Clin Toxicol 50:231–241

Le Quesne PM, Goldberg V, Vajda F (1976) Acute conduction velocity changes in guinea-pigs after administration of diphenylhydantoin. J Neurol Neurosurg Psychiatry 39:995–1000

Lee KC, Ladizinski B, Federman DG (2012) Complications associated with use of levamisole-contaminated cocaine: an emerging public health challenge. Mayo Clin Proc 87:581–586

LeGatt DF (2009) New cocaine cutting agent poses greater risk to users. Clinical & Forensic Toxicology News, Sep 2009

Leichman L, Brown T, Poplin B (1993) Symptomatic, radiologic and pathologic changes in the central nervous system (CNS) associated with 5-fluorouracil (5-FU) and levamisole (LEV) therapy. Proc Am Soc Clin Oncol 12:198

Levandoski MM, Piket B, Chang J (2003) The anthelmintic levamisole is an allosteric modulator of human neuronal nicotinic acetylcholine receptors. Eur J Pharmacol 471:9–20

Lewis JA, Wu CH, Levine JH, Berg H (1980) Levamisole-resistant mutants of the nematode Caenorhabditis elegans appear to lack pharmacological acetylcholine receptors. Neuroscience 5:967–989

Liu HM, Hsieh WJ, Yang CC, Wu VC, Wu KD (2006) Leukoencephalopathy induced by levamisole alone for the treatment of recurrent aphthous ulcers. Neurology 67:1065–1067

Lott IT, Coulombe T, Di Paolo RV, Richardson EP Jr, Levy HL (1978) Vitamin B6-dependent seizures: pathology and chemical findings in brain. Neurology 28:47–54

Lucchinetti CF, Kimmel DW, Pavelko K, Rodriguez M (1997) 5-Fluorouracil and levamisole exacerbate demyelination in susceptible mice infected with Theiler's virus. Exp Neurol 147:123–129

Lucia P, Pocek M, Passacantando A, Sebastiani ML, De Martinis C (1996) Multifocal leucoencephalopathy induced by levamisole. Lancet 348:1450

Luyckx M, Cazin M, Brunet C, Cazin JC, Devulder B, Gosselin P, Adenis L, Cappelaere P, Granier AM, Demaille A (1983) Lymphocytes T and pharmacokinetics after a single oral dose of levamisole in healthy and cancer subjects. Methods Find Exp Clin Pharmacol 5:467–469

Macdonald RL, Kang JQ, Gallagher MJ (2010) Mutations in $GABA_A$ receptor subunits associated with genetic epilepsies. J Physiol 588:1861–1869

Macfarlane DG, Bacon PA (1978) Levamisole-induced vasculitis due to circulating immune complexes. Br Med J 1(6110):407–408

Marcus DJ, Swift TR, McDonald TF (1981) Acute effects of phenytoin on peripheral nerve function in the rat. Muscle Nerve 4:48–50

Martin DL, Rimvall K (1993) Regulation of gamma-aminobutyric acid synthesis in the brain. J Neurochem 60:395–407

Matute C, Torre I, Pérez-Cerdá F, Pérez-Samartín A, Alberdi E, Etxebarria E, Arranz AM, Ravid R, Rodríguez-Antigüedad A, Sánchez-Gómez M, Domercq M (2007) P2X(7) receptor blockade prevents ATP excitotoxicity in oligodendrocytes and ameliorates experimental autoimmune encephalomyelitis. J Neurosci 27:9525–9533

McCormick DA (1989) GABA as an inhibitory neurotransmitter in human cerebral cortex. J Neurophysiol 62:1018–1027

Mehansho H, Henderson LM (1980) Transport and accumulation of pyridoxine and pyridoxal by erythrocytes. J Biol Chem 255:11901–11907

Millán JL (2006). Mammalian alkaline phosphatases. From biology to applications in medicine and biotechnology. Wiley-VCH Verlag GmbH & Co, Weinheim, Germany

Mitzdorf U (1985) Current source-density method and application in cat cerebral cortex: investigation of evoked potentials and EEG phenomena. Physiol Rev 65:37–100

Moertel CG, Fleming TR, Macdonald JS, Haller DG, Laurie JA, Goodman PJ, Ungerleider JS, Emerson WA, Tormey DC, Glick JH, Veerder MH, Mailliard JA (1990) Levamisole and fluorouracil for adjuvant therapy of resected colon carcinoma. N Engl J Med 322:352–358

Moertel CG, Fleming TR, Macdonald JS, Haller DG, Laurie JA, Tangen CM, Ungerleider JS, Emerson WA, Tormey DC, Glick JH, Veeder MH, Mailliard JA (1995) Fluorouracil plus levamisole as effective adjuvant therapy after resection of stage III colon carcinoma: a final report. Ann Int Med 122:321–326

Mohammad FK, Faris GA, Rhayma MS, Ahmed K (2006) Neurobehavioral effects of tetramisole in mice. Neurotoxicology 27:153–157

Mongeau JG, Robitaille PO, Roy F (1988) Clinical efficacy of levamisole in the treatment of primary nephrosis in children. Pediatr Nephrol 2:398–401

Mori S, Nagano M (1985) Electron microscopic cytochemistry of alkaline phosphatase in neurons of rats. Arch Histol Jpn 48:389–397

Morley SR, Forrest ARW, Galloway JH (2006) Levamisole as a contaminant of illicit Cocaine. Int Assoc Forensic Toxicologists 44:6

Mornet E (2007) Hypophosphatasia. Orphanet J Rare Dis 2:40

Morris DC, Masuhara K, Takaoka K, Ono K, Anderson HC (1992) Immunolocalization of alkaline phosphatase in osteoblasts and matrix vesicles of human fetal bone. Bone Miner 19:287–298

Murray CL, Ford WJ, Swenson KK, Heros D, Sperduto PW (1997) Multifocal inflammatory leukoencephalopathy after fluorouracil and levamisole therapy for colon cancer. AJNR Am J Neuroradiol 18:1591–1592

Nandy K, Bourne GH (1963) Alkaline phosphatases in brain and spinal cord. Nature 200:1216–1217

Narisawa S, Fröhlander N, Millán JL (1997) Inactivation of two mouse alkaline phosphatase genes and establishment of a model of infantile hypophosphatasia. Dev Dyn 208:432–446

Narisawa S, Wennberg C, Millán JL (2001) Abnormal vitamin B6 metabolism in alkaline phosphatase knock-out mice causes multiple abnormalities, but not the impaired bone mineralization. J Pathol 193:125–133

Négyessy L, Xiao J, Kántor O, Kovács GG, Palkovits M, Dóczi TP, Renaud L, Baksa G, Glasz T, Ashaber M, Barone P, Fonta C (2011) Layer-specific activity of tissue non-specific alkaline phosphatase in the human neocortex. Neuroscience 172:406–418

Neu IS (1993) Multifocal inflammatory leukencephalopathy caused by adjuvant therapy with 5-fluorouracil and levamisole after resection for an adenocarcinoma of the colon. Acta Neurol Scand 87:70

Niaudet P, Drachman R, Gagnadoux MF, Broyer M (1984) Treatment of idiopathic nephrotic syndrome with levamisole. Acta Paediatr Scand 73:637–641

Nowak LG, Bullier J (1996) Spread of stimulating current in the cortical grey matter of rat visual cortex studied on a new in vitro slice preparation. J Neurosci Methods 67:237–248

Ohkubo S, Kimura J, Matsuoka I (2000) Ecto-alkaline phosphatase in NG108-15 cells : a key enzyme mediating P1 antagonist-sensitive ATP response. Br J Pharmacol 131:1667–1672

Onuaguluchi G, Igbo IN (1987) Comparative local anaesthetic and antiarrhythmic actions of levamisole hydrochloride and lignocaine hydrochloride. Arch Int Pharmacodyn Ther 289:278–289

Pabst HF, Crawford J (1975) L-tetramisole. Enhancement of human lymphocyte response to antigen. Clin Exp Immunol 21:468–473

Pagonopoulou O, Efthimiadou A, Asimakopoulos B, Nikolettos NK (2006) Modulatory role of adenosine and its receptors in epilepsy: possible therapeutic approaches. Neurosci Res 56:14–20

Palcoux JB, Niaudet P, Goumy P (1994) Side effects of levamisole in children with nephrosis. Pediatr Nephrol 8:263–264

Parkinson DR, Cano PO, Jerry LM, Capek A, Shibata HR, Mansell PW, Lewis MG, Marquis G (1977) Complications of cancer immunotherapy with levamisole. Lancet 1(8022):1129–1132

Paterson D, Nordberg A (2000) Neuronal nicotinic receptors in the human brain. Prog Neurobiol 61:75–111

Percudani R, Peracchi A (2009) The B6 database: a tool for the description and classification of vitamin B6-dependent enzymatic activities and of the corresponding protein families. BMC Bioinformatics 10:273

Perry DC, Xiao Y, Nguyen HN, Musachio JL, Dávila-García MI, Kellar KJ (2002) Measuring nicotinic receptors with characteristics of alpha4beta2, alpha3beta2 and alpha3beta4 subtypes in rat tissues by autoradiography. J Neurochem 82:468–481

Picher M, Burch LH, Hirsh AJ, Spychala J, Boucher RC (2003) Ecto 5'-nucleotidase and nonspecific alkaline phosphatase. Two AMP-hydrolyzing ectoenzymes with distinct roles in human airways. J Biol Chem 278:13468–13479

Pinner B, Davison JF, Campbell JB (1964) Alkaline Phosphatase in Peripheral Nerves. Science 145:936–938

Pinto V, Derkach VA, Safronov BV (2008) Role of TTX-sensitive and TTX-resistant sodium channels in Adelta- and C-fiber conduction and synaptic transmission. J Neurophysiol 99:617–628

Pires JG, Futuro-Neto HA, Oliveira AM, Cabral AM (1979) The effect of levamisole on the cardiac responses to sympathomimetics and on the amine uptake process. J Pharm Pharmacol 31:257–258

Prieur AM, Buriot D, Lefur JM, Griscelli C (1978) Possible toxicity of levamisole in children with rheumatoid arthritis. J Pediatr 93:304–305

Raeymaekers AH, Allewijn FT, Vandenberk J, Demoen PJ, Van Offenwert TT, Janssen PA (1966) Novel broad-spectrum anthelmintics. Tetramisole and related derivatives of 6-arylimidazo [2,1-b]thiazole. J Med Chem 9:545–551

Rathbun JC (1948) Hypophosphatasia; a new developmental anomaly. Am J Dis Child 75:822–831

Rayes D, De Rosa MJ, Bartos M, Bouzat C (2004) Molecular basis of the differential sensitivity of nematode and mammalian muscle to the anthelmintic agent levamisole. J Biol Chem 279:36372–36381

Raymond SA (1992) Subblocking concentrations of local anesthetics: effects on impulse generation and conduction in single myelinated sciatic nerve axons in frog. Anesth Analg 75:906–921

Rehni AK, Singh TG (2010) Levamisole-induced reduction in seizure threshold: a possible role of nicotinic acetylcholine receptor-mediated pathway. Naunyn Schmiedebergs Arch Pharmacol 382:279–285

Reid JM, Kovach JS, O'Connell MJ, Bagniewski PG, Moertel CG (1998) Clinical and pharmacokinetic studies of high-dose levamisole in combination with 5-fluorouracil in patients with advanced cancer. Cancer Chemother Pharmacol 41:477–484

Renoux G, Renoux M (1972) Antigenic competition and nonspecific immunity after a rickettsial infection in mice: restoration of antibacterial immunity by phenyl-imidothiazole treatment. J Immunol 109:761–765

Renoux G, Renoux M, Teller MN, McMahon S, Guillaumin JM (1976) Potentiation of T-cell mediated immunity by levamisole. Clin Exp Immunol 25:288–296

Rifkin DB, Compans RW, Reich E (1972) A specific labeling procedure for proteins on the outer surface of membranes. J Biol Chem 247:6432–64327

Robertson SJ, Martin RJ (1993) Levamisole-activated single-channel currents from muscle of the nematode parasite Ascaris suum. Br J Pharmacol 108:170–178

Ruuskanen O, Remes M, Mäkela AL, Isomäki H, Toivanen A (1976) Levamisole and agranulocytosis. Lancet 308:958–959

Sanchez MR (2000) Miscellaneous treatments: thalidomide, potassium iodide, levamisole, clofazimine, colchicine, and D-penicillamine. Clin Dermatol 18:131–145

Sanchez-Vives MV, McCormick DA (2000) Cellular and network mechanisms of rhythmic recurrent activity in neocortex. Nat Neurosci 3:1027–1034

Savarese DM, Gordon J, Smith TW, Litofsky NS, Licho R, Ragland R, Recht L (1996) Cerebral demyelination syndrome in a patient treated with 5-fluorouracil and levamisole. The use of thallium SPECT imaging to assist in noninvasive diagnosis—a case report. Cancer 77:387–394

Scheinberg MA, Bezerra JB, Almeida FA, Silveira LA (1978) Cutaneous necrotising vasculitis induced by levamisole. Br Med J 1(6110):408

Schiller JH, Lindstrom M, Witt PL, Hank JA, Mahvi D, Wagner RJ, Sondel P, Borden EC (1991) Immunological effects of levamisole in vitro. J Immunother 10:297–306

Schneider S, Meys F (2011) Analysis of illicit cocaine and heroin samples seized in Luxembourg from 2005 to 2010. Forensic Sci Int 212:242–246

Schuermans Y (1975) Levamisole in rheumatoid arthritis. Lancet 1(7898):111

Schwarz SK, Puil E (1998) Analgesic and sedative concentrations of lignocaine shunt tonic and burst firing in thalamocortical neurones. Br J Pharmacol 124:1633–1642

Seidlin M, Straus SE (1984) Treatment of mucocutaneous herpes simplex infections. Clin Dermatol 2:100–116

Selzer ME (1979) The effect of phenytoin on the action potential of a vertebrate spinal neuron. Brain Res 171:511–521

Sergienko EA, Millán JL (2010) High-throughput screening of tissue-nonspecific alkaline phosphatase for identification of effectors with diverse modes of action. Nat Protoc 5:1431–1439

Sharp AJ, Polak PE, Simonini V, Lin SX, Richardson JC, Bongarzone ER, Feinstein DL (2008) P2x7 deficiency suppresses development of experimental autoimmune encephalomyelitis. J Neuroinflammation 5:33

Shimizu N (1950) Histochemical studies on the phosphatase of the nervous system. J Comp Neurol 93:201–217

Simão AM, Bolean M, Hoylaerts MF, Millán JL, Ciancaglini P (2013) Effects of pH on the production of phosphate and pyrophosphate by matrix vesicles' biomimetics. Calcif Tissue Int 93:222–232

Spector R (1978) Vitamin B6 transport in the central nervous system: in vivo studies. J Neurochem 30:881–887

Spector S, Munjal I, Schmidt DE (1998) Effects of the immunostimulant, levamisole, on opiate withdrawal and levels of endogenous opiate alkaloids and monoamine neurotransmitters in rat brain. Neuropsychopharmacology 19:417–427

Sperlágh B, Illes P (2014) P2X7 receptor: an emerging target in central nervous system diseases. Trends Pharmacol Sci 35:537–547

Spreafico F, Vecchi A, Mantovani A, Poggi A, Franchi G, Anaclerio A, Garattini S (1975) Characterization of the immunostimulants levamisole and tetramisole. Eur J Cancer 11:555–563

Stevens B, Porta S, Haak LL, Gallo V, Fields RD (2002) Adenosine: a neuron-glial transmitter promoting myelination in the CNS in response to action potentials. Neuron 36:855–868

Street SE, Kramer NJ, Walsh PL, Taylor-Blake B, Yadav MC, King IF, Vihko P, Wightman RM, Millán JL, Zylka MJ (2013) Tissue-nonspecific alkaline phosphatase acts redundantly with PAP and NT5E to generate adenosine in the dorsal spinal cord. J Neurosci 33:11314–11322

Stys PK (2013) Pathoetiology of multiple sclerosis: are we barking up the wrong tree? F1000Prime Rep 5:20

Sugimura K, Mizutani A (1979) Histochemical and cytochemical studies of alkaline phosphatase activity in the synapses of rat brain. Histochemistry 61:123–129

Suzuki K, Yoshimura Y, Hisada Y, Matsumoto A (1994) Sensitivity of intestinal alkaline phosphatase to L-homoarginine and its regulation by subunit-subunit interaction. Jpn J Pharmacol 64:97–102

Symoens J, Veys E, Mielants M, Pinals R (1978) Adverse reactions to levamisole. Cancer Treat Rep 62:1721–1730

Taketani T, Onigata K, Kobayashi H, Mushimoto Y, Fukuda S, Yamaguchi S (2014) Clinical and genetic aspects of hypophosphatasia in Japanese patients. Arch Dis Child 99:211–215

Tanphaichitr P, Tanphaichitr D, Sureeratanan J, Chatasingh S (1980) Treatment of nephrotic syndrome with levamisole. J Pediatr 96:490–493

Thienpont D, Vanparijs OF, Raeymaekers AH, Vandenberk J, Demoen JA, Allewijn FT, Marsboom RP, Niemegeers CJ, Schellekens KH, Janssen PA (1966) Tetramisole (R 8299), a new, potent broad spectrum anthelmintic. Nature 209:1084–1086

Toivanen A, Lassila O, Nordman E (1981) Lack of effect of levamisole on the immune function in melanoma patients. Cancer Immunol Immunother 10:191–195

Trautmann A (2009) Extracellular ATP in the immune system: more than just a “danger signal”. Sci Sig 2:pe6

Treurniet-Donker AD, Meischke-de Jongh ML, van Putten WL (1987) Levamisole as adjuvant immunotherapy in breast cancer. Cancer 59:1590–1593

Tsutsui S, Schnermann J, Noorbakhsh F, Henry S, Yong VW, Winston BW, Warren K, Power C (2004) A1 adenosine receptor upregulation and activation attenuates neuroinflammation and demyelination in a model of multiple sclerosis. J Neurosci 24:1521–1529

van Belle H (1972) Kinetics and inhibition of alkaline phosphatases from canine tissues. Biochim Biophys Acta 289:158–168

van Belle H (1976a) Kinetics and inhibition of rat and avian alkaline phosphatases. Gen Pharmacol 7:53–58

van Belle H (1976b) Alkaline phosphatase. I. Kinetics and inhibition by levamisole of purified isoenzymes from humans. Clin Chem 22:972–976

van der Ham M, Albersen M, de Koning TJ, Visser G, Middendorp A, Bosma M, Verhoeven-Duif NM, de Sain-van der Velden MG (2012) Quantification of vitamin B6 vitamers in human cerebrospinal fluid by ultra performance liquid chromatography-tandem mass spectrometry. Anal Chim Acta 712:108–114

Vandevelde M, Boring JG, Hoff EJ, Gingerich DA (1978) The effect of levamisole on the canine central nervous system. J Neuropathol Exp Neurol 37:165–173

Vanhoutte PM, Van Nueten JM, Verbeuren TJ, Laduron PM (1977) Differential effects of the isomers of tetramisole on adrenergic neurotransmission in cutaneous veins of dog. J Pharmacol Exp Ther 200:127–140

Waymire KG, Mahuren JD, Jaje JM, Guilarte TR, Coburn SP, MacGregor GR (1995) Mice lacking tissue non-specific alkaline phosphatase die from seizures due to defective metabolism of vitamin B-6. Nat Genet 11:45–51

Webster DJ, Whitehead RH, Richardson G, Hughes LE (1982) Levamisole: a double blind immunological study. Anticancer Res 2:29–32

Whyte MP (2010) Physiological role of alkaline phosphatase explored in hypophosphatasia. Ann NY Acad Sci 1192:190–200

Whyte MP, Mahuren JD, Fedde KN, Cole FS, McCabe ER, Coburn SP (1988) Perinatal hypophosphatasia: tissue levels of vitamin B6 are unremarkable despite markedly increased circulating concentrations of pyridoxal-5′-phosphate. Evidence for an ectoenzyme role for tissue-nonspecific alkaline phosphatase. J Clin Invest 81:1234–1239

Whyte MP, Mahuren JD, Vrabel LA, Coburn SP (1985) Markedly increased circulating pyridoxal-5′-phosphate levels in hypophosphatasia. Alkaline phosphatase acts in vitamin B6 metabolism. J Clin Invest 76:752–756

Wiebke EA, Grieshop NA, Loehrer PJ, Eckert GJ, Sidner RA (2003) Antitumor effects of 5-fluorouracil on human colon cancer cell lines: antagonism by levamisole. J Surg Res 111:63–69

Woestenborghs R, Michielsen L, Heykants J (1981) Determination of levamisole in plasma and animal tissues by gas chromatography with thermionic specific detection. J Chromatogr 224:25–32

Wu VC, Huang JW, Lien HC, Hsieh ST, Liu HM, Yang CC, Lin YH, Hwang JJ, Wu KD (2006) Levamisole-induced multifocal inflammatory leukoencephalopathy: clinical characteristics, outcome, and impact of treatment in 31 patients. Medicine 85:203–213

Yao SQ, Li ZZ, Huang QY, Li F, Wang ZW, Augusto E, He JC, Wang XT, Chen JF, Zheng RY (2012) Genetic inactivation of the adenosine A(2A) receptor exacerbates brain damage in mice with experimental autoimmune encephalomyelitis. J Neurochem 123:100–112

Yokota T, Saito Y, Miyatake T (1994) Conduction slowing without conduction block of compound muscle and nerve action potentials due to sodium channel block. J Neurol Sci 124:220–224

Zakon HH (2012) Adaptive evolution of voltage-gated sodium channels: the first 800 million years. Proc Natl Acad Sci USA 109(Suppl 1):10619–10625

Zhang D, Xiong W, Chu S, Sun C, Albensi BC, Parkinson FE (2012) Inhibition of hippocampal synaptic activity by ATP, hypoxia or oxygen-glucose deprivation does not require CD73. PLoS ONE 7:e39772

Zimmermann H, Zebisch M, Sträter N (2012) Cellular function and molecular structure of ecto-nucleotidases. Purinergic Signal 8:437–502