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Lobeline inhibits Ca^{2+} current in cultured neurones from rat sympathetic ganglia

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Abstract

The effect of lobeline was studied on the voltage-activated Ca^{2+} current in sympathetic neurones from the rat superior cervical ganglia using the whole-cell variant of the patch–clamp technique. Lobeline (10–300 μM) inhibited the Ca^{2+} current evoked by voltage steps from −80 mV (holding potential) to 0 mV (test potential) in a dose dependent manner. The inhibitory effects of noradrenaline (10 μM) and lobeline (100 μM) were compared using a prepulse protocol with high (+80 mV) depolarization. Within the same cell depolarizing prepulses decreased the inhibitory effect of noradrenaline but did not change the extent of lobeline inhibition. Addition of GTPγS (300 μM) to the pipette solution did not prevent the inhibitory effect of lobeline (100 μM) but greatly reduced that of noradrenaline (100 μM). Our experiments suggest, that the weak nicotinic agonist lobeline exerts a direct blocking effect on Ca^{2+} channels at concentrations commonly used to release transmitters.

Keywords: Lobeline; Nicotinic receptor agonist; Ca^{2+} channel; Patch clamp; Sympathetic neurone, rat

1. Introduction

Nicotine and nicotinic acetylcholine receptor agonists are frequently used to evoke transmitter release from both central and peripheral nervous system preparations (Arqueros et al., 1978; Niebler and Trendelenburg, 1990; Todorov et al., 1991; Vizi et al., 1995; Sacaan et al., 1995; Kiss et al., 1997; Kulak et al., 1997). It has been suggested, that nicotinic acetylcholine receptor agonists can increase Ca^{2+}-dependent transmitter release by directly activating Ca^{2+} permeable nicotinic acetylcholine receptors at the release sites (Muelle et al., 1992; Vizi et al., 1995; Sershen et al., 1997; Wonnacott, 1997) or by inducing axonal firing, and the action potentials depolarize the nerve terminals where Ca^{2+} necessary for transmitter release enters into the cell via the voltage-sensitive Ca^{2+} channels (Sershen et al., 1997; Wonnacott, 1997). The latter mechanism can be blocked by inhibitors of voltage-dependent Na^{+} channels (by preventing action potentials; Sershen et al., 1997), with blockers of certain Ca^{2+} channel subtypes (Vaughan et al., 1993; Vizi et al., 1995; Soliakov et al., 1995; Soliakov and Wonnacott, 1996; Sershen et al., 1997) or by noradrenaline (Vizi et al., 1995; Kiss et al., 1997).

Lobeline is a high-affinity ligand on the nicotinic acetylcholine receptor (Lippiello and Fernandes, 1986) and its transmitter releasing action is often compared to other nicotinic agonists in a variety of preparations. One of the hallmarks of the nicotinic action is that the effect of the agonists can be antagonized by mecamylamine on somatodendritic (Vizi et al., 1995; Vizi and Lendvai, 1997) and on presynaptic nicotinic acetylcholine receptors (cf. Wonnacott, 1997). Another important characteristic of nicotinic action on transmitter release is that it is Ca^{2+}-dependent (cf. Wonnacott, 1997). However, one of the main findings of the experiments in which the transmitter-releasing action of lobeline was compared to nicotine was, that lobeline evoked transmitter release is Ca^{2+} independent and mecamylamine insensitive (Clarke and Reuben, 1996; Teng et al., 1997). It has also been shown that lobeline-induced serotonin release was inhibited by cooling the tissue to 7°C, suggesting that the lobeline-evoked release was mediated by a carrier protein (Lendvai et al., 1996; Vizi, 1998).

These experiments clearly demonstrate that lobeline induces transmitter release by a mechanism other than

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activation of nicotine receptors. But it still remains obscure why the Ca^{2+}-dependent and mecamylamine-sensitive component of the release (which is expected from the nicotinic agonistic action of lobeline) can not be detected. The lack of nicotinic receptor activation-dependent transmitter release can be explained if lobeline inhibits one or more components of the Ca^{2+}-dependent transmitter liberation. This hypothesis is further supported by the recent finding of Rao et al. (1997), who demonstrated that *N*-methyl-D-aspartate evoked striatal [^{3}H]acetylcholine release is attenuated by lobeline.

In our experiments we investigated whether lobeline can inhibit the voltage-dependent Ca^{2+} channels, which action might serve as a possible explanation for the inability of lobeline to evoke transmitter release through the nicotinic receptor activation.

2. Materials and methods

Superior cervical ganglia from 1–3-day-old postnatal Wistar rats were isolated according to the method of Higgins et al. (1991). Sympathetic neuron cell cultures were prepared as described previously (Toth and Miller, 1995). Ca^{2+} current measurements were made from sympathetic neurones grown on poly-L-lysine treated glass coverslips within 48 h after isolation. Voltage-sensitive Ca^{2+} current was recorded in the whole-cell configuration of the patch clamp technique (Hamill et al., 1981) at room temperature (21°–24°C). The pipette solution contained (in mM): CsCl 100, CsOH 37, $MgCl_2$ 1, 1,2-bis(2-aminophenoxy)-ethane-*N*,*N*,*N*′,*N*′-tetraacetic acid (BAPTA) 10, Hepes 10, MgATP 3.6, phosphocreatine (di-tris salt; 14), GTP 1 and creatine phosphokinase, 50 units/ml. In one series of experiments GTPγS (0.3 mM) was also included (GTP: GTPγS = 10: 3) in the pipette solution. The extracellular solution contained (in mM): $CaCl_2$ 2, tetraethylammonium chloride 160, $MgCl_2$ 1, Hepes 10 and glucose 10; the pH was adjusted to 7.4 with tetraethylammonium hydroxide for the isolation of inward Ca^{2+} current. Pipettes were made from borosilicate glass (Sutter Instrument) and had resistance in the range of 1.8–3 MΩ. Rectangular test pulses (200-ms duration) were delivered every 20 s from the holding potential of −80 mV to the test potential of 0 mV. In one set of experiments (as described in Fig. 5) test pulses were 30 ms long. The waveform of the applied prepulse protocol is described at the appropriate figure legend. Whole-cell currents were recorded with an Axopatch-1D amplifier (Axon Instruments). Current traces were filtered at 2 kHz and sampled at 5 kHz. Series resistance was routinely compensated by about 70%–80%. Leak current was subtracted by using the P/4 protocol. Recordings were made with bath perfusion; drug application was carried out by switching to a drug-containing bath solution.

The effects of the noradrenaline and lobeline were calculated by comparing the peak value of the control Ca^{2+} current before the treatment to the magnitude of the inhibited current at the same time point. Data are represented as mean ± standard error of the mean (S.E.M.). Tetraethylammonium chloride, l-lobeline, l-noradrenaline, pipette solution and culture media constituents were purchased from Sigma.

3. Results

The effect of the high concentration of lobeline was tested on voltage-activated Ca^{2+} channels in cultured neurones from the rat sympathetic ganglia. Lobeline (300 μM) inhibited the whole-cell Ca^{2+} current (Fig. 1) in a reversible manner. The inhibition developed quickly and the inhibited current returned to the initial control level upon switching back to the control solution (Fig. 1A). Desensitization did not develop to the drug; subsequent application of lobeline was equally effective (Fig. 1A, C). The effect of lobeline was characterized in the 10–300 μM concentration range (Fig. 2). Nearly 60% inhibition of

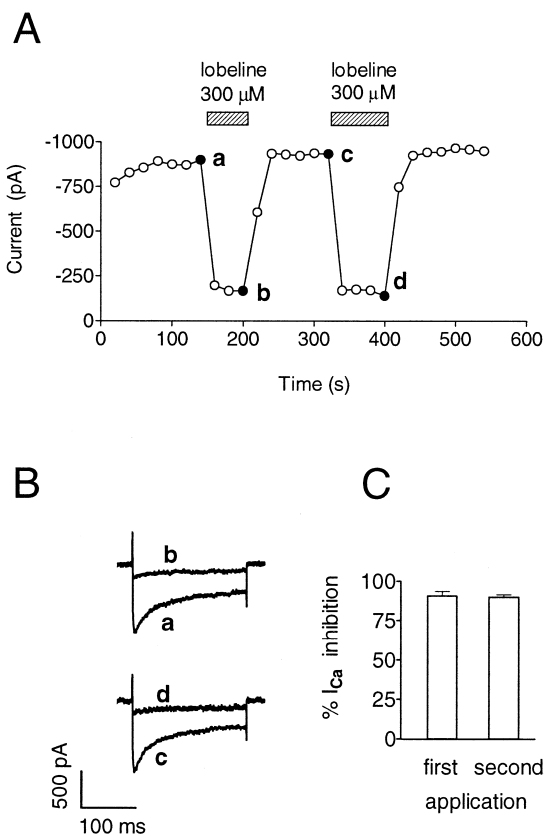

Fig. 1. Inhibition of whole-cell I_{Ca} by lobeline (300 μM). Whole-cell I_{Ca} elicited by 200 ms depolarizing test pulses to 0 mV from a holding potential of −80 mV. Interpulse interval was 20 s. (A) Plot of peak current vs. time. (B) Leak subtracted currents show the inhibition at time points indicated in (A). (C) Histograms represent the mean percent inhibition of the whole-cell peak I_{Ca} by two subsequent applications of 300 μM lobeline ($n = 5$). The vertical lines represent the S.E.M..

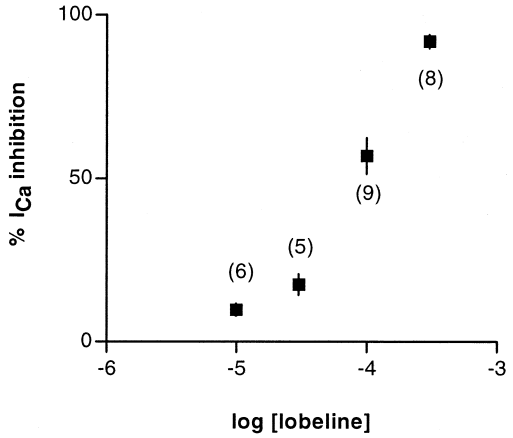

Fig. 2. Dose–response characteristics of peak whole-cell I_{Ca} inhibition by lobeline. Data were pulled together from all experiments, except from those where lobeline and noradrenaline effects were tested on cells dyalized with GTPγS (i.e., Fig. 5). 100 μM lobeline produced 56.9% inhibition of the whole-cell peak I_{Ca}.

Ca^{2+} current was achieved by 100 μM lobeline (56.9 ± 5.59%; mean ± S.E.M.; $n = 9$); therefore, this concentration was chosen for the further experiments. The current–voltage relationship (Fig. 3) revealed a shift of the peak and the apparent reversal potential of the inhibited current toward more negative potentials. The current–voltage relationship also displayed a substantial inhibitory effect of lobeline at the very positive voltages. Inhibition of Ca^{2+} channels by certain neurotransmitters display different characteristics. In the presence of inhibitory neurotransmitters, very high depolarizations, or application of a depolarizing prepulse immediately before the test pulse can relieve the channel block produced by the neurotransmitter. In sympathetic neurones, and also in other preparations, a large depolarizing prepulse applied immediately before the test pulse has been shown to remove a large part

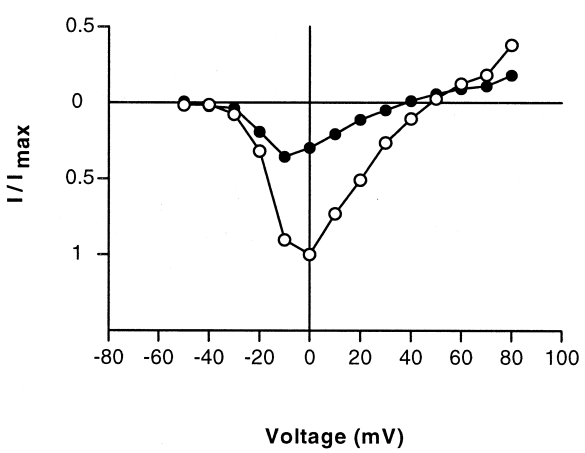

Fig. 3. The $I-V$ relationships recorded from holding potential of −80 mV to test potentials of −50 to +80 mV before (○) and during exposure (•) to lobeline (300 μM). Corresponding peak current values from three identical experiments were averaged, and normalized to the maximal value.

of the transmitter-mediated inhibition of Ca^{2+} current (Elmslie et al., 1990; Zhu and Ikeda, 1994). Therefore, we compared the characteristics of current inhibition by noradrenaline and lobeline in the same cell. The applied stimulation template (Fig. 4A) contained a prepulse to a very high voltage (+80 mV). The effect of 10 μM

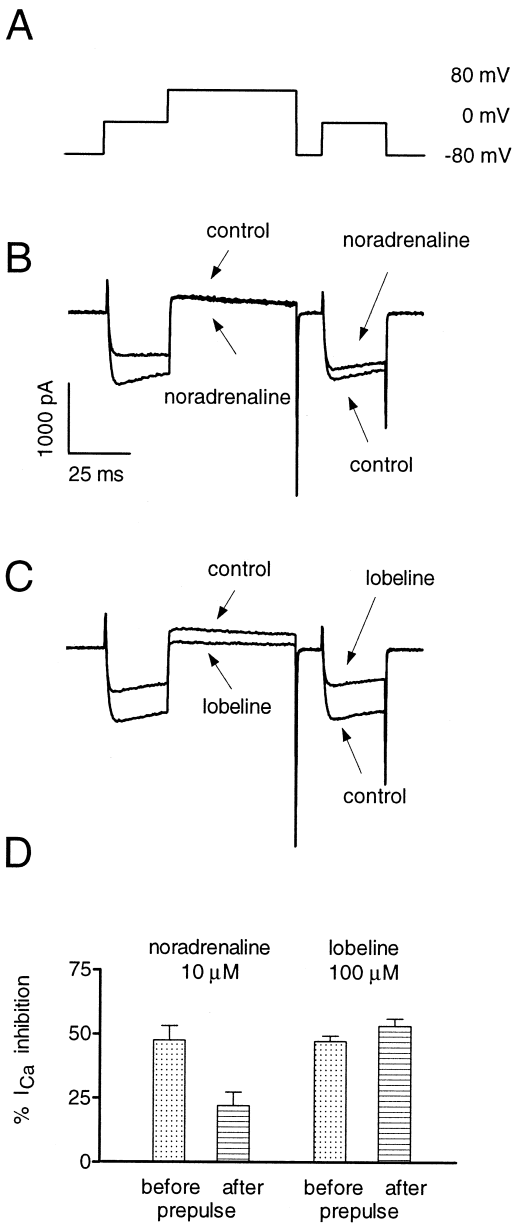

Fig. 4. Voltage dependence of I_{Ca} inhibition by noradrenaline and lobeline. (A) Voltage waveform used in this series of experiments contained a 50 ms depolarization to +80 mV immediately before the second test pulse. Test pulses to 0 mV were 25 ms long. (B) Control and inhibited current traces during 10 μM noradrenaline application. (C) Control and inhibited current traces during 100 μM lobeline application. Scale bar on B applies to both B and C. (D) Quantitative results from 5 cells. Ordinate shows percent inhibition of I_{Ca}. Control and inhibited current values from individual cells were taken at the time point where peak I_{Ca} was observed under control conditions. The vertical lines represent the S.E.M..

noradrenaline and 100 μM lobeline is shown in Fig. 4B, C. The inhibitory effect of noradrenaline was diminished after the depolarization to +80 mV (Fig. 4B, D; 47.6 ± 5.6% inhibition before, 22.1 ± 5.3% after the depolarization to +80 mV; $n = 5$, mean ± S.E.M.; $P < 0.05$). Note also, that the outward Cs^+ current which can be seen during the depolarization to +80 mV, was not inhibited. In contrast, lobeline retained its blocking effect after the large depolarization (Fig. 4C, D; 47.2 ± 2.1% inhibition before, 53.2 ± 2.9% inhibition after the depolarization to +80 mV; $n = 5$, mean ± S.E.M.). The outward current during the predepolarization was also inhibited (Fig. 4C), which is consistent with the inhibition at positive voltages shown in the current–voltage relationship (Fig. 3). Since G-protein-mediated Ca^{2+} current inhibition can also happen in a voltage-independent manner, we further compared the effects of noradrenaline and lobeline in cells dialyzed with GTPγS (GTP:GTPγS ratio 10:3). Noradrenaline (100 μM) and lobeline (100 μM) were applied to separate cells at the same time point, at 1000 s after the beginning of the experiment (Fig. 5B). The inhibition of Ca^{2+} current by non-specific activation of G-proteins by GTPγS was 43.2 ± 4.2% (n = 9). Noradrenaline application after this period did not substantially block the remaining Ca^{2+} current (5.1 ± 1.7% inhibition, $n = 4$). The small amount of inhibition we have seen can probably be attributed to the large amount of GTP we had in the patch pipette. However, lobeline still blocked the remaining current (53.6 ± 2.5% inhibition; $n = 5$; mean ± S.E.M.) under these conditions (Fig. 5A). These experiments also confirm that the effect of lobeline on Ca^{2+} channels is G-protein independent.

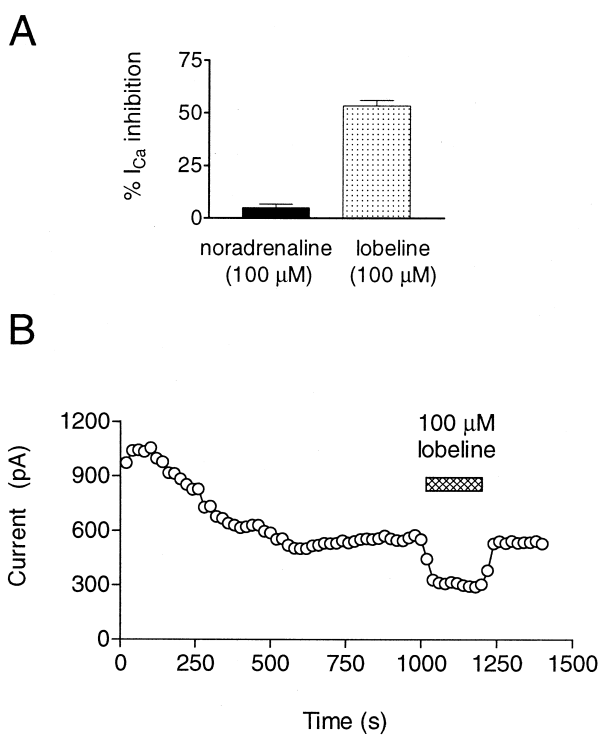

Fig. 5. Lobeline does not act via G-protein-channel coupling. (A) Permanent activation of G-proteins by GTPγS (300 μM) is sufficient to inhibit Ca^{2+} channel modulation by 100 μM noradrenaline (5.12 ± 1.67% inhibition; $n = 4$). GTPγS (300 μM) failed to prevent the inhibitory effect of lobeline (53.60 ± 2.59% inhibition; $n = 5$). Histogram represents percent inhibition of peak I_{Ca} in cells dialyzed with GTPγS (300 μM) together with GTP (1 mM). (B) Time course of a representative experiment displaying the effect of lobeline (100 μM). Lobeline and noradrenaline were applied to different cells at the same time point (1000 s). Test pulses (30 ms long) were applied from −80 mV to 0 mV every 20 s. Empty symbols represents current values at the time point where peak current was observed at the beginning of the experiment.

4. Discussion

Voltage-dependent Ca^{2+} channels play a major role in the Ca^{2+}-dependent processes in neurones, such as transmitter release (cf. Miller, 1988). Apart from the well-known blockers of the neuronal Ca^{2+} channels (specific toxins, di- and trivalent cations, etc.; see Dolphin, 1995), Ca^{2+} channels can be inhibited nonselectively by a large number of other drugs, for example, dopamine receptor antagonists (Sah and Bean, 1994) used in relatively high concentrations. In our experiments, we found that lobeline, used in the high micromolar concentration range also blocks Ca^{2+} channels in sympathetic neurones of the rat.

Lobeline exerts a weak nicotinic agonist action in a variety of preparations. In comparison with other nicotinic agonists (cytisine, nicotine, dimethylphenyl-piperazinium, acetylcholine, carbachol) lobeline proved to be the least potent nicotinic agonist to evoke inward current via nicotinic receptor activation in sympathetic neurones from the rat superior ganglia (Covernton et al., 1994). Because of its lower efficiency, lobeline as a nicotinic agonist is often used in the 50–300 μM concentration range to evoke transmitter release (Clarke and Reuben, 1996; Lendvai et al., 1996; Sershen et al., 1997; Teng et al., 1997). However, transmitter release evoked by high concentrations of lobeline does not share the same characteristics as the transmitter release evoked by the other nicotinic agonists. The lobeline-evoked transmitter release proved to be mecamylamine insensitive, the time course of transmitter release (Clarke and Reuben, 1996; Teng et al., 1997) and the efficacy of lobeline differs from that of nicotine (Clarke and Reuben, 1996; Sershen et al., 1997, Teng et al., 1997).

In the present study lobeline was found to inhibit the voltage-dependent Ca^{2+} channels in rat sympathetic neurones. In 300 μM (the highest concentration tested) lobeline almost completely abolished (92% inhibition) the voltage-gated Ca^{2+} current. In rat sympathetic neurones different types of Ca^{2+} channels contribute to the whole-cell Ca^{2+} current; however, 80%–90% of the current flows through the N-type Ca^{2+} channels (Regan et al., 1991; Mintz et al., 1992; Toth and Miller, 1995). In this case the inhibition of a single type of Ca^{2+} channel could

not account for the observed $92 \pm 2.1\%$ inhibition, so probably more than one type of Ca^{2+} channel was affected by high concentrations of the drug.

The current–voltage relationship of lobeline inhibition highlighted several important points. The apparent reversal potential and the peak of the current–voltage relationship were shifted toward more negative potentials. Since we used CsCl in our pipette solution, it is conceivable that lobeline alters the P_{Ca}/P_{Cs} ratio. However, we have not carried out experiments with an impermeable cation in the pipette to fully analyze this phenomenon, or to reveal the details of the lobeline effect on the alteration of membrane surface charge and channel gating. Instead, we have focused on another observation, the inhibitory effect of lobeline observed at higher depolarizations.

In the rat sympathetic neurones a large number of neurotransmitters and hormones are able to inhibit Ca^{2+} channels; including noradrenaline, acetylcholine, somatostatin, angiotensin II, substance P, pancreatic polypeptide and vasoactive intestinal peptide (Hille, 1994, and references therein). Large concentrations of lobeline might have the ability to affect any of these receptors and inhibit Ca^{2+} channels through receptor-mediated G-protein interaction. A large number of the G-protein-dependent inhibitory pathway are voltage dependent, but not all of them (Hille, 1994). The current–voltage relationship of lobeline inhibition demonstrated a marked inhibition at the very positive voltages. This observation suggested that lobeline does not utilize the G-protein-mediated voltage-dependent inhibitory pathway to block Ca^{2+} channels. To test this possibility we compared the inhibitory effects of lobeline and noradrenaline on the same cell. Prepulse voltage protocols are often used to demonstrate the G-protein-mediated voltage-dependent inhibitory effects of various neurotransmitters (Dolphin, 1996). Predepolarization of the membrane potential just prior to the subsequent test pulse removes a large part of the Ca^{2+} current inhibition by neurotransmitters (Elmslie et al., 1990; Zhu and Ikeda, 1994). Consistent with these results, we have observed the relief of inhibition following high depolarization in the case of noradrenaline. The inhibitory potency of lobeline remained unchanged, or even a little increase could be seen after the prepulse. These results demonstrated that lobeline did not inhibit Ca^{2+} current in a voltage-dependent manner like that of noradrenaline. The lack of voltage-dependent nature of the Ca^{2+} current inhibition by lobeline can not completely exclude the role of other G-protein-mediated pathways in the drug's effect. Neurotransmitters can inhibit Ca^{2+} channels by several different mechanisms. Even a single transmitter can utilize more than just one pathway (Hille, 1994). Although the inhibitory pathways are G-protein mediated, there are some differences between them (i.e., pertussis toxin, voltage, and Ca^{2+} sensitivity; Hille, 1994). Therefore, the possible role of G-proteins in the lobeline effect was further investigated with a more general approach, including putting GTPγS into the pipette solution. Irreversible activation of all types of G-proteins inhibited the response to noradrenaline. On the other hand GTPγS did not prevent the inhibitory action of lobeline on the Ca^{2+} current. The most plausible explanation for this finding is, that lobeline has a direct blocking effect on the Ca^{2+} channels.

There are two main findings of the recent work. We have added a new member to the list of drugs known to inhibit Ca^{2+} channels by demonstrating the inhibitory effect of lobeline on the voltage-dependent Ca^{2+} channels. Secondly, we suggest, that voltage-operated N-type Ca^{2+} channels do not play a role in the transmitter release evoked by high concentrations of lobeline.

Nicotinic acetylcholine receptor agonists can evoke transmitter release via the activation of the nicotinic receptor and/or through some nonspecific action. Until recently drugs which displayed high affinity to the nicotinic acetylcholine receptors were used to evoke transmitter release without full characterization of their mechanism of action. The transmitter releasing action was attributed to the increase of free intracellular Ca^{2+} which was a result of the nicotinic acetylcholine receptor activation. Neuronal nicotinic receptors are permeable to both Na^{+} and Ca^{2+}. Ca^{2+}-influx into the axon terminal can directly activate transmitter release (Wonnacott, 1997). Na^{+}-influx through nicotinic acetylcholine receptor can depolarize the varicose arborisation to a certain threshold where it starts to fire action potentials. During action potentials Ca^{2+} enters into the varicosities through voltage sensitive Ca^{2+} channels. Lobeline, as a nicotinic acetylcholine receptor agonist might drive the cells into the firing state, but the drug itself can block Ca^{2+} entry into the cells by inhibiting voltage dependent Ca^{2+} channels and as a consequence, Ca^{2+}-dependent transmitter release. The question remained, where the transmitter releasing effect of lobeline comes from? Recent data from the literature suggest, that the transmitter releasing action of lobeline might not be Ca^{2+} and nicotinic acetylcholine receptor dependent (Clarke and Reuben, 1996; Teng et al., 1997). A carrier protein mediated release process might also play a role in the mechanism of action of lobeline (Lendvai et al., 1996).

Demonstration of the possible effect of lobeline on the other type of Ca^{2+} channels requires further experiments. Since lobeline has been used as a substitution therapy for smoking cessation (cf. Olin et al., 1995) its Ca^{2+} channel blocking property may be able to explain some of its side effects observed during treatment.

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