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Pharmacological characterization of amine receptors on embryonic chick sensory neurones

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1 The effects of noradrenaline, dopamine and 5-hydroxytryptamine were investigated on the duration of the action potential of embryonic chick sensory neurones *in vitro*.

2 All three amines, like γ-aminobutyric acid, decreased the duration of the action potential evoked by current injection.

3 The onset of the noradrenaline-induced decrease in action potential duration was fast (< 1 s) and the recovery phase was dependent upon the dose of noradrenaline applied. Rapid washout of the noradrenaline revealed a minimum 30 s recovery time which was independent of the initial noradrenaline concentration.

4 Dopamine and 5-hydroxytryptamine could mimic the effects of noradrenaline on action potential duration. The ED_{50} for all three amines was approximately 1 μM. At a saturating concentration of 10 μM, noradrenaline was more potent than dopamine and 5-hydroxytryptamine.

5 Saturating doses of noradrenaline and dopamine or 5-hydroxytryptamine were not additive.

6 Responses to all three amines were affected similarly by antagonists: they were antagonized by yohimbine, phentolamine, haloperidol and mianserin but not by propranolol, prazosin, domperidone, spiperone or methysergide. Clonidine and xylazine (α_2-adrenoceptor agonists) were also without effect.

7 In contrast to the amines, saturating concentrations of γ-aminobutyric acid were additive with those of noradrenaline. Responses to GABA were not antagonized by the amine receptor antagonists.

8 The evidence described here suggests that the amines and γ-aminobutyric acid decrease sensory neurone action potential duration via pharmacologically-distinct membrane receptors. In addition, it is likely that the amines are acting via a single class of receptor whose pharmacology is different from classical adrenoceptors, dopamine receptors and 5-hydroxytryptamine receptors.

Introduction

Embryonic chick sensory neurones *in vitro* are sensitive to noradrenaline (NA): NA evokes a reduction in Ca^{2+} influx through the voltage-dependent Ca channel in the soma membrane of these neurones (Dunlap & Fischbach, 1978; 1981). Similar effects were also observed for γ-aminobutyric acid (GABA) (Dunlap, 1981).

Antagonism of the NA-induced decrease in Ca^{2+} current by phentolamine suggested that an α-adrenoceptor was involved. This was supported by the observation that phenylephrine could mimic the effects of NA on the Ca action potential (Dunlap & Fischbach, 1978). In contrast, phentolamine does not block the response to GABA, suggesting that NA and GABA act via separate membrane receptors. The purpose of the experiments described here was to examine in greater detail the pharmacology of this response to NA and to investigate further the relationship of the NA and GABA receptors on these cells. Some of these results have appeared in preliminary form (Dunlap & Canfield, 1983).

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Method

Sensory neurones were dissected from 11–12 day old chicken embryos, mechanically dissociated into single cells, and grown *in vitro* using methods previously described (Dunlap & Fischbach, 1978; 1981). The growth medium contained Dulbecco's modified

Minimum Essential Medium, 10% horse serum, 5% chick embryo extract, 1 mM glutamine, 1 $\mu g\,ml^{-1}$ 7 s Nerve Growth Factor, 50 u ml^{-1} penicillin and 50 $\mu g\,ml^{-1}$ streptomycin. Standard intracellular recording and current-passing techniques were employed to study the effects of NA on the cultured neurones between 5 days and 2 months *in vitro*. Membrane potentials were monitored with 30–40 MΩ microelectrodes filled with 3 M KCl and current was passed through the recording electrode via an active bridge circuit. Recording medium contained (in mM): NaCl 131, KCl 5.9, $CaCl_2$ 5, $MgCl_2$ 0.8, and N-2-hydroxyethylpiperazine-N-2-ethanesulphonic acid (HEPES) 25, pH 7.4. Drugs, dissolved in recording medium were applied by pressure (2–5 psi) ejection from blunt-tipped (3–5 μm) pipettes positioned $<50\,\mu$m from the impaled neurone. Control experiments have shown that this technique results in less than a 10% dilution of the pipette (puffer) solution and therefore offers a rapid and reversible means of applying known drug concentrations to individual neurones (Choi, 1978; Choi & Fischbach, 1981). Action potential duration was measured directly from the storage oscilloscope screen as the time from peak to half amplitude on the falling phase. Noradrenaline ((±)-Arterenol), 5-hydroxytryptamine (5-HT), 3-hydroxytyramine, yohimbine and γ-aminobutyric acid were obtained from Sigma. The other drugs were obtained from the sources indicated as follows: haloperidol, domperidone, spiperone (Janssen), mianserin (Organon), clonidine (Boehringer-Ingelheim, Ltd.), phentolamine (CIBA-Geigy), xylazine (Cutter).

Results

Agonistic effects of the amines and GABA

NA and GABA decreased the duration of the soma action potential (Figure 1c). The onset of the decrease in action potential duration (APD) is relatively rapid, effects being observed within 1 s (the approximate limit of resolution with puffer application of drugs), and maximal effects being achieved within approximately 5 s. The time-course for the return to control was found to be a function of NA concentration if, following a 5 s application, the transmitter was allowed to diffuse passively away from the cell (Figure 1a). The half time for return to control was 15, 25, and 100 s for 1, 10 and 100 μM NA, respectively. If, on the other hand, NA was washed out with a continuous application of recording medium immediately following the application of NA, the recovery time was not only dramatically reduced but also became independent of the concentration of NA (Figure 1b). In this case, the half time for recovery was 15 s for all three concentrations. One prominent feature of this drug-induced decrease in APD was that it did not 'desensitize' in the maintained presence of the drug.

The effects of NA on APD could be mimicked by GABA, dopamine and 5-HT (Dunlap & Fischbach, 1978; Dunlap, 1981). The dose-response curves in figure 2 illustrate that NA, dopamine, 5-HT and GABA were all effective over the concentration range of $>10^{-8}$ to 10^{-5} M, with ED_{50}s of about 1 μM. At the saturating dose of 10 μM, NA and GABA were more potent than either dopamine or 5-HT, producing average maximal decreases in APD of 42%(NA) and 45%(GABA) compared to 30% for dopamine and 28% for 5-HT. Application of supersaturating doses ($>10\,\mu$M) of the amines produced smaller responses than those elicited by maximal doses.

Maximal doses of NA and GABA, when applied together, produced additive effects (Figure 3). In this experiment a supersaturating concentration of NA (100 μM) was first applied to a sensory neurone soma while the action potential was being continuously monitored. Once the maximal effect of NA was obtained, 100 μM GABA (also saturating) was applied (in the maintained presence of NA) from a second puffer. Additional decreases in APD were observed following the application of GABA. Similar results were obtained when the drugs were applied in the reverse order.

Decreases in APD produced by dopamine or by 5-HT were not additive with those produced by NA. Using two puffer pipettes, one containing 10 μM NA, the other 10 μM NA plus either 10 μM dopamine or 10 μM 5-HT, (saturating concentrations of all drugs, see Figure 2) the average percentage decrease in APD was monitored following the initial application of NA and subsequent application of the combined drugs. For the NA/dopamine additivity experiment, NA produced an average maximum decrease in APD of $46 \pm 8\%$ (±s.e.mean, $n = 10$). Subsequent application of NA + dopamine produced no further decrease in APD in any of these 10 cells. In contrast, application of NA + dopamine + GABA (via the second puffer) resulted in a total decrease in APD of $64 \pm 6\%$ ($n = 10$). Similar results were obtained in NA/5-HT additivity experiments. NA applied alone produced an average $39 \pm 3\%$ decrease in APD while NA + 5-HT produced no further decrease in APD in any of the cells ($n = 17$). Addition of GABA to the solution applied via the second puffer, however, resulted in a total decrease in APD of $50 \pm 8\%$ ($n = 4$). That is, unlike GABA, neither dopamine nor 5-HT produced any further decreases in APD over that produced by NA alone.

To study the dose-dependence of the effect of NA on the dopamine or 5-HT-induced decrease in APD,

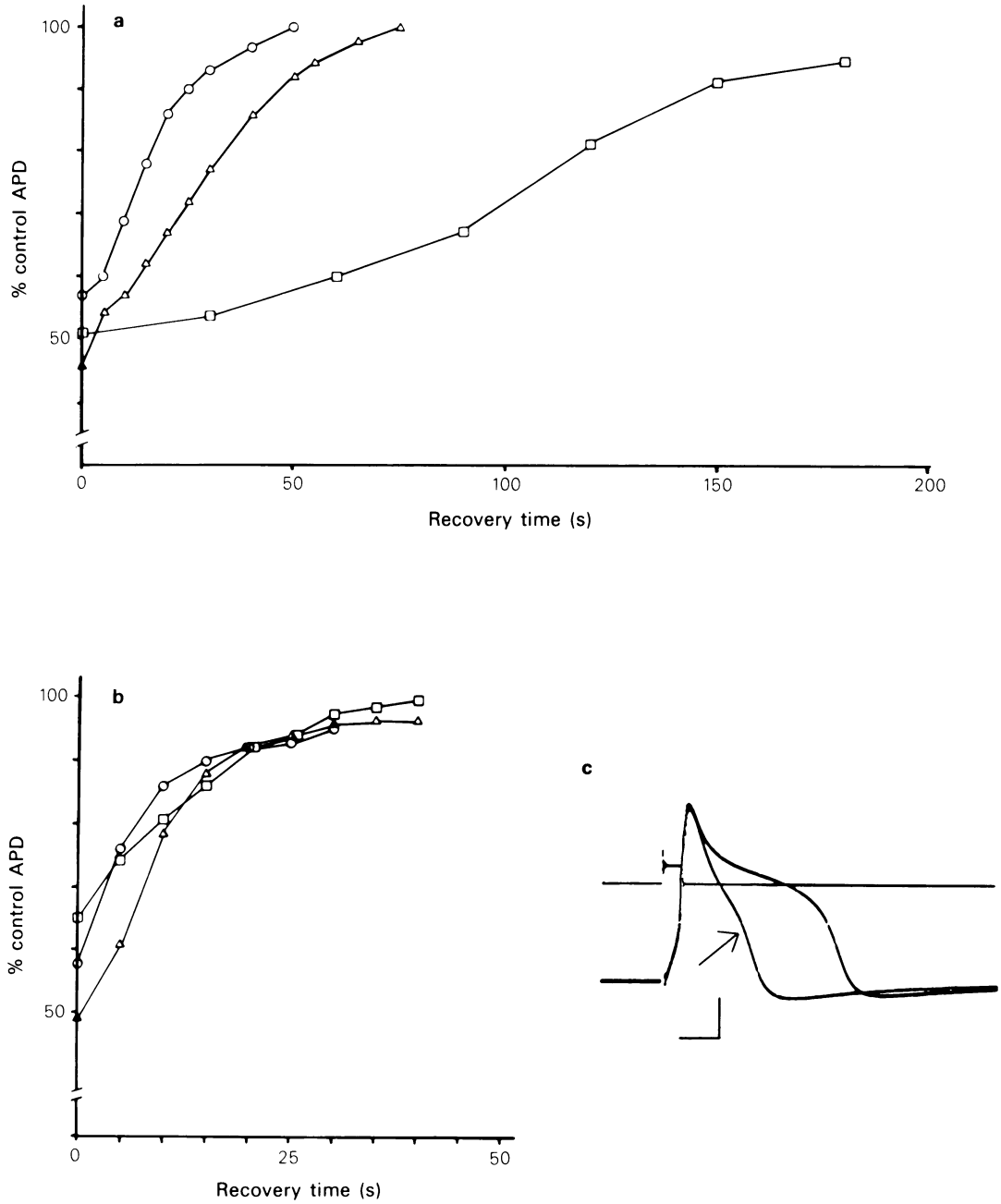

Figure 1 Effect of noradrenaline (NA) on action potential duration (APD) in embryonic chick sensory neurones. (a) and (b) Average time-courses (3 cells per curve) for action potential duration recovery following 5 s application of 1 μM (○), 10 μM (△) and 100 μM (□) NA under conditions in which (a) the NA was allowed to diffuse passively away from the cell or (b) the NA was rapidly washed away by pressure application of recording medium from second pipette. (c) Two superimposed oscilloscope traces of a soma action potential before and after (arrow) application of 10 μM NA (70% decrease in APD). Upper trace shows the depolarizing current pulse applied to the cell. Cal: 20mV, 50nA and 2ms.

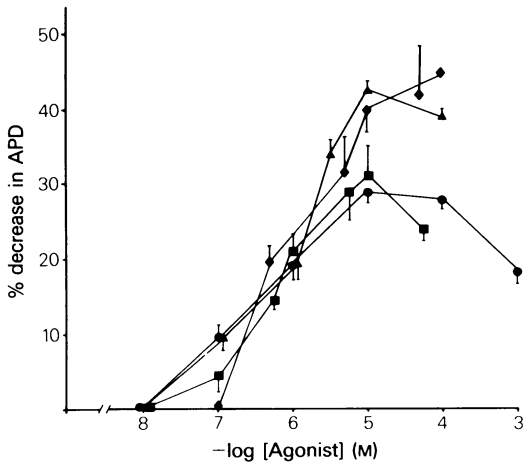

Figure 2 The effects of noradrenaline (NA), dopamine, 5-hydroxytryptamine (5-HT), and γ-aminobutyric acid (GABA) on the duration of the action potential duration (APD) in embryonic chick sensory neurones. Ordinate scale: percentage decrease in action potential duration produced by various concentrations of NA (▲), dopamine (●), 5-HT (■) and GABA (◆); abscissa scale: agonist concentration. Each point is the mean of results from a minimum of 10 cells with s.e.mean indicated by vertical lines. APD was calculated as the time from peak to half amplitude on the falling phase.

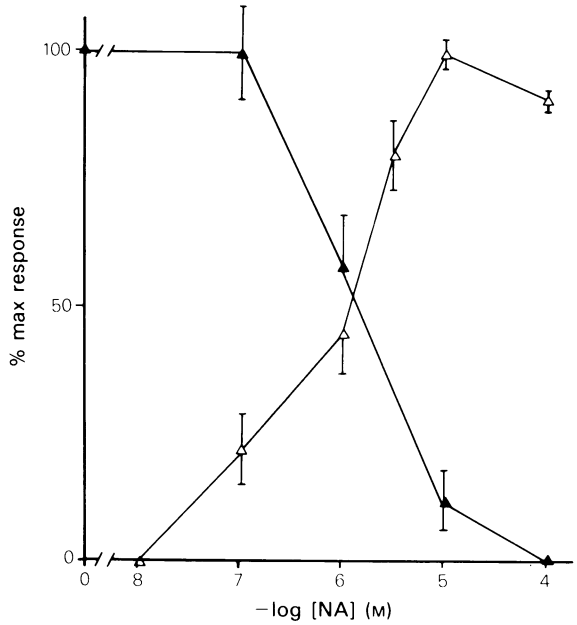

Figure 4 Effects of noradrenaline (NA) on the duration of the action potential (APD) in embryonic chick sensory neurones and on the decrease in APD evoked by dopamine. Ordinate scale: percentage of maximal response evoked by either agonist; abscissa scale: concentration of NA. (△) NA alone; (▲) dopamine + NA. For the latter, the cells were incubated in NA prior to dopamine application.

the additivity experiment described above was performed with the following alterations. The maximum percentage decrease in APD produced by 10 μM dopamine was recorded in 10 neurones. NA, in various concentrations, was then added to the recording bath and the dopamine puffer. The magnitude of the dopamine effect was then assayed in 10 different neurones at each NA concentration. The lack of desensitization of the amine effect on APD allowed for the maintained action of NA in this experiment. The inhibitory dose-response curve is shown in Figure 4. The ID_{50} for the antagonism of the response to dopamine by NA was very similar to the ED_{50} observed for the agonistic activity of NA.

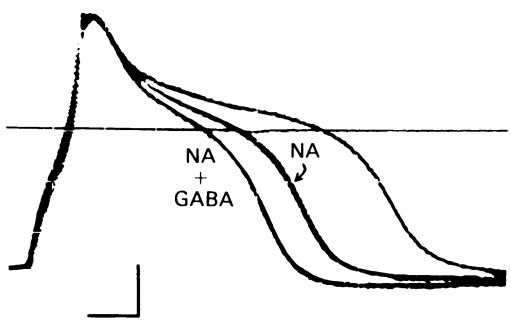

Figure 3 Additive effect of noradrenaline (NA) and γ-aminobutyric acid (GABA) on action potential duration. Three superimposed action potentials recorded from a sensory neurone cell body before and during application of supersaturating concentrations of 100 μM NA (NA) or 100 μM GABA plus 100 μM NA (NA + GABA). Calibration: 20mV, 2ms.

Similar results were obtained under equilibrium conditions in which the transmitters were added directly to the bathing solution. In this case the amines were applied in the reverse order from that described above. The ED_{50} for the dopamine-induced decrease in APD was 0.7 μM and the ID_{50} for the antagonism of the response to NA by dopamine was 0.8 μM. As before, GABA was still able to produce decreases in APD in neurones incubated in saturating concentrations of NA and/or dopamine.

Effects of antagonists

Phentolamine, an α-adrenoceptor antagonist, blocked the response to NA in a dose-dependent fashion with an ID_{50} of 0.16 μM (Figure 5). Phentolamine also antagonized the dopamine- and 5-HT-mediated decreases in APD but not those mediated by GABA (not shown). The β-adrenoceptor antagonist, prop-

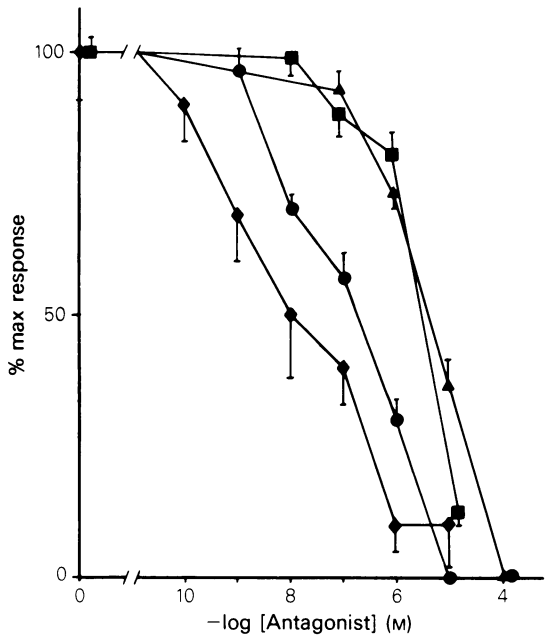

Figure 5 Antagonism of the noradrenaline (NA)-induced decrease in action potential duration by phentolamine (●), haloperidol (◆), clonidine (▲) and mianserin (■). Ordinate scale: percentage maximal response to NA; abscissa scale: concentration of antagonists.

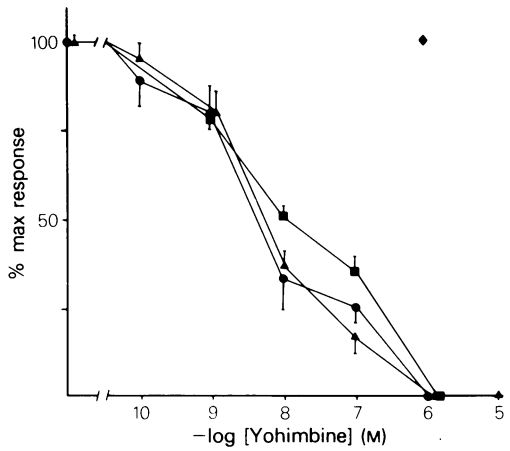

Figure 6 The effect of yohimbine on the reduction in action potential duration evoked by noradrenaline (▲), dopamine (●), 5-hydroxytryptamine (■) and GABA (◆). Ordinate scale: percentage of maximal response to the agonists; abscissa scale: concentration of yohimbine. Each point is the mean of results from a minimum of 10 different neurones with s.e.mean indicated by vertical lines. Note that while responses to the amines were equally antagonized, the response to GABA remained unaffected by yohimbine.

ranolol, in concentrations as high as 10 μM, did not block the effects of the amines. As previously reported, 10 μM phenylephrine, but not 10 μM isoprenaline mimicked the response to NA (Dunlap & Fischbach, 1978). In addition, yohimbine, an α_2-adrenoceptor antagonist (Kobinger, 1978), was a potent inhibitor of NA-, dopamine and 5-HT-induced decreases in APD (Figure 6). The ID_{50} for the antagonism of the responses to all of the agonists was between 5 and 10 nM. The dose-response relationship for the antagonism of responses by yohimbine was virtually identical for all three amines. In contrast, yohimbine did not block responses to GABA. In the presence of yohimbine 1 μM (a concentration which antagonized responses of these same cells to the amines), GABA decreased APD by $30 \pm 2\%$ compared to $30 \pm 1.3\%$ in the absence of yohimbine ($n = 7$ for each group).

The specific α_2-adrenoceptor agonists clonidine and xylazine (Kobinger, 1978), in concentrations ranging between 0.1 and 100 μM, did not decrease APD ($n > 20$). Indeed, in high concentration clonidine was seen to inhibit the NA-induced decrease in APD (Figure 5). Prazosin (a specific α_1-adrenoceptor antagonist) was ineffective in blocking NA-induced decreases in APD at all concentrations tested (0.1–100 μM, $n > 10$ at each concentration).

The dopamine receptor antagonists haloperidol, domperidone and spiperone were also tested for their effectiveness in antagonizing the amine-induced changes in APD. Haloperidol (0.1 nM to 10 μM) (Figure 5), but not domperidone (10 μM) or spiperone (10 μM), was able to antagonize the NA-induced decrease in APD; the ID_{50} for haloperidol was 10 nM. The 5-HT antagonist mianserin (0.01–100 μM) (Figure 5), but not methysergide (10 μM), also antagonized the NA-induced decrease in APD with an ID_{50} of 3 μM. This antagonism by mianserin required relatively high concentrations compared to those required for yohimbine and haloperidol. Responses to dopamine and 5-HT (but not those to GABA) were also inhibited by the same concentrations of the dopamine and 5-HT antagonists which blocked responses to NA.

Discussion

The results described here are consistent with the notion that the decrease in sensory neurone action potential duration produced by the amines is mediated by membrane receptors pharmacologically distinct from those involved in GABA-induced changes in APD on these same cells: yohimbine, a potent inhibitor of the amine-induced decreases in APD, was ineffective in antagonizing responses to GABA. The effects on APD of saturating concentra-

tions of NA and GABA were additive. Results of voltage clamp experiments have shown that these two transmitters decreased sensory neurone Ca current by an identical mechanism (Dunlap & Fischbach, 1981). It is possible, therefore, that the additive effect of NA and GABA on APD results from their action on two separate populations of receptor which ultimately activate the same mechanism to decrease APD.

Similar experiments using NA, dopamine and 5-HT suggest that these amines may not be acting via separate membrane receptors. In contrast to the experiments with GABA, saturating concentrations of any two amines applied together were not additive. If amine-induced decreases in APD involve the same mechanism as those for GABA, then this lack of additive effect suggests that NA, dopamine and 5-HT all act via the same receptor. The similarity of the ED_{50} for NA's agonistic activity and the ID_{50} for its antagonistic effect on the dopamine-induced decreases in APD (Figure 4), further supports this notion of a single amine receptor. The possibility cannot be excluded, however, that the amines act via separate membrane receptors, and the lack of additive effect results from saturation of a common step removed from the initial drug-receptor interaction.

The observations with the antagonists also support the notion that the amines act via the same receptor. The dose-dependent antagonism by yohimbine is virtually identical for all three amines. In addition, all antagonists which were effective in blocking responses to NA, also blocked dopamine- and 5-HT-mediated responses, and the agents ineffective in antagonizing responses to NA were also ineffective on responses to dopamine and 5-HT. The wide range of antagonists tested, taken together with their apparent inability to discriminate between responses to the three amines, further suggests that NA, dopamine and 5-HT all act via the same receptor.

Functional effects of NA similar to those described here, have been observed on adult sympathetic ganglion neurones of the rat (Horn & McAfee, 1979; 1980; Galvan & Adams, 1982). Dopamine can mimic these effects of NA and phentolamine antagonizes them. Inhibition of noradrenaline release from sympathetic nerve terminals by α-adrenoceptor agonists is well-established (Starke, 1977). It is possible that both the modulation of Ca^{2+} current and the block of transmitter release from sympathetic neurones are mediated by a similar receptor.

In sensory neurones, NA-induced decreases in Ca-dependent action potential duration may have important implications for modulation of synaptic efficacy between sensory neurones and spinal cord cells. NA is thought to be involved in the inhibition of sensory neurotransmitter release in the dorsal horn of the spinal cord of the rat (Jeftinija *et al.*, 1982). Little is known about the pharmacology of this receptor, but autoradiographic studies show specific binding sites for [^{3}H]-yohimbine in this same region of the spinal cord (Young & Kuhar, 1979). The Ca-dependent release of substance P (a putative sensory neurotransmitter) from chick sensory neurones *in vitro* can be blocked by NA, an effect that is antagonized by phentolamine (Mudge, 1979). If the effects of the amines on cell body Ca action potentials described here parallel their effects on sensory nerve terminals in the spinal cord, then the NA-mediated inhibition of sensory neurotransmitter release may result from an action of NA on the voltage-dependent Ca channel in the nerve terminal.

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