Br. J. Pharmacol. (1991), **104**, 159–165

Substance P modulates the time course of nicotinic but not muscarinic catecholamine secretion from perfused adrenal glands of rat

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1 Substance P (SP) and acetylcholine (ACh) are contained within the splanchnic nerve terminals in the adrenal gland and can be released in response to stress. In the rat, the release of ACh brings about secretion of catecholamines (CA) by acting on nicotinic and muscarinic receptors on the adrenal chromaffin cells.

2 In the present study, we have used a rat isolated adrenal gland preparation to investigate the effects of SP, perfused at different concentrations, on CA secretion evoked by 10^{-5} M nicotine and 10^{-4} M muscarine.

3 In the first 10 min stimulation period (S1), in the absence of SP, nicotine (10^{-5} M) evoked substantial and equal secretion of noradrenaline (NA) and adrenaline (Ad). In a second 10 min stimulation period (S2), carried out 18 min after S1, the nicotinic response was desensitized. In contrast, the muscarinic response, which preferentially evoked Ad secretion in S1 (Ad/NA: 8.7/1), was well maintained in S2.

4 SP present in S1 had no effect on desensitization of the subsequent nicotinic response in S2.

5 At low concentrations (10^{-7}–10^{-10} M), SP changed the time course of nicotine-induced CA secretion during S1 by enhancing CA secretion in the first 4 min and inhibiting CA secretion thereafter. The maximal effect occurred at 10^{-9} M SP.

6 At a higher concentration (10^{-5} M), SP inhibited total nicotinic CA secretion throughout S1 and produced a biphasic secretion of CA (depressed in the presence of SP and enhanced after wash out of SP). Pre-exposure of adrenal glands to SP (10^{-9} to 10^{-5} M) for 10 min produced marked inhibition of the nicotine-induced CA secretion.

7 In contrast to the effect of SP on the nicotinic response, SP from 10^{-9} to 10^{-5} M had no effect on muscarinic CA secretion.

8 This difference in sensitivity of the nicotinic and muscarinic responses to SP points to a diversity of mechanisms available for control of adrenal catecholamine secretion. In addition to the ability of SP to increase or decrease the total amount of adrenal CA secretion, dependent on the concentration of SP, the present study shows that SP can change the time-course of nicotinic CA secretion. These results with the rat adrenal gland perfused *in vitro* suggests both a quantitative and temporal role for SP as a novel modulator of adrenal CA secretion.

Keywords: Substance P; adrenal medulla; noradrenaline; adrenaline; nicotine; catecholamine; muscarine; neuromodulation

Introduction

Catecholamine (CA) secretion from the adrenal gland is mediated by acetylcholine (ACh) released from splanchnic nerve terminals innervating the adrenal medulla (Feldberg *et al.*, 1934). In most mammals, ACh activates both nicotinic and muscarinic receptors to evoke secretion (Feldberg *et al.*, 1934; Douglas & Poisner, 1965; Critchley *et al.*, 1986; for review see Livett, 1987). In recent years, substantial evidence has accumulated indicating that, in addition to the classical neurotransmitter ACh, a large number of endogeneous neuropeptides are involved in the regulation of CA secretion either as neurotransmitters or as neuromodulators (for review, see Marley & Livett, 1985; Livett, 1987).

Substance P (SP)-like immunoreactivity has been identified in nerve terminals innervating the adrenal medulla of man (Linnoila *et al.*, 1980), pig (Kong *et al.*, 1989), and rat (Kuramoto *et al.*, 1987). In the rat, SP nerves in the adrenal medulla originate from the sensory neurones in the ipsilateral dorsal root ganglia rather than cholinergic motor neurones in the spinal cord (Zhou *et al.*, 1990). In addition, SP can be released *in vivo* from the adrenal medulla in response to physiological stress (Vaupel *et al.*, 1988). Bovine adrenal medullary membranes have specific high affinity binding sites for SP (Geraghty *et al.*, 1990) that are distinct from the classical NK_1, NK_2 and NK_3 receptors in other systems. However, the possible functional role of SP in adrenal medullary secretion remains unclear.

Functional studies indicate that SP has no effect on the basal secretion or veratridine- or potassium-induced secretion from bovine isolated chromaffin cells (Livett *et al.*, 1979; Boksa & Livett, 1984a). However, SP does inhibit nicotine- or ACh-evoked CA secretion (Livett *et al.*, 1979; Role *et al.*, 1981), and preincubation with SP can protect against desensitization of the nicotinic response (Boksa & Livett, 1984b; Khalil *et al.*, 1988a). In contrast to these studies in chromaffin cells in which SP protected against desensitization of the nicotinic response, in PC12 cells, a rat phaeochromocytoma cell line, SP enhanced desensitization of the nicotinic response as measured by sodium influx in response to nicotinic agonists (Stallcup & Patrick, 1980; Simasko *et al.*, 1985; 1987). More recently, Boyd & Leeman (1987) reported that SP treatment of PC12 cells in the absence of agonist caused the nicotinic acetylcholine receptor to become subsequently non-responsive to agonists.

The rat adrenal medulla possesses both functional nicotinic and muscarinic receptors (Wakade, 1981). Previous studies in perfused rat adrenal glands have shown that SP modulates adrenal CA secretion evoked by electrical field stimulation (Zhou & Livett, 1990b). However, the involvement of nicotinic vs muscarinic responses in this process has not been determined. SP is the most potent mammalian tachykinin for modulating nicotinic receptor function (Khalil *et al.*, 1988b; Boyd & Leeman, 1987). Therefore, in the present study, we

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have used rat isolated perfused adrenal glands and studied: (1) the effect of SP on CA secretion evoked by nicotine and muscarine; (2) the effect of SP on the desensitization of the nicotinic response; and (3) the effect of pre-exposure to SP on the subsequent nicotinic CA secretion. We found that SP had multiple effects on nicotinic CA secretion but had no effect on muscarinic-induced CA secretion.

Methods

Preparation of the perfused adrenal gland

Adult male and female Buffalo rats, 250 to 350 g, were used throughout. The rats were anaesthetized with Equithesin (a mixture of pentobarbitone and chloral hydrate, i.p., 2.6 ml kg^{-1}, for detail, see Tagerud & Cuello, 1979) and an L-shaped incision was made on the left side of the abdomen. The left adrenal gland was isolated and perfused essentially as described by Wakade (1981). In this preparation the adrenal vein was cannulated rather than the renal vein to reduce leakage from collaterals (Zhou & Livett, 1990b). After cannulation, the gland was isolated from the rat, put in a heated chamber (circulated with 37°C water) and perfused at 125 μl min^{-1} with Krebs bicarbonate solution, consisting of (mM): NaCl 118.4, $NaHCO_3$ 25, KCl 4.7, $CaCl_2$ 2.5, $MgCl_2$ 1.18, KH_2PO_4 1.2 and glucose 11.7. The perfusion solution was gassed with 95% O_2 and 5% CO_2 to a final pH of 7.4.

Effect of substance P on nicotine-evoked catecholamine secretion

For testing the effect of SP on the nicotinic CA secretion, two protocols were used. In one protocol (Figures 1, 2 and 3), CA release was evoked by perfusing with 10^{-5} M nicotine for two 10 min periods, separated by an 18 min rest period. In the first stimulation period (S1), SP at different concentrations (0–10^{-5} M) was perfused into the adrenal gland for 2 min before and 10 min during the nicotine perfusion. In the second stimulation period (S2), SP was omitted to test the effect of SP present in the first stimulation on desensitization of nicotine-induced CA secretion in the second stimulation. The perfusate was collected every 2 min for CA assay.

In the second protocol (Figure 4), the adrenal gland was stimulated once by perfusion with 10^{-5} M nicotine. SP (10^{-9} to 10^{-5} M) was perfused for 10 min before and for 10 min during perfusion with 10^{-5} M nicotine.

Effect of substance P on muscarine-evoked catecholamine secretion

For testing the effect of SP on muscarinic CA secretion (Figures 5 and 6), adrenal glands were stimulated twice by perfusion with 100 μM muscarine for 10 min. In S1, only 100 μM muscarine was perfused; in S2, SP at different concentrations was perfused for 2 min before and 10 min during perfusion with 100 μM muscarine. There was an 18 min interval between the two stimulations. The perfusate was collected every 2 min for CA assay.

Measurement of endogeneous catecholamines

The perfusates were acidified with perchloric acid (PCA, 0.4 M final concentration) and stored at −20°C until assay. For determination of noradrenaline (NA) and adrenaline (Ad), the perfusate was centrifuged at 4000 *g* for 10 min at 2°C. The supernatant was mixed with 3,4-dihydroxybenzylamine (DHBA, 2 nmol ml^{-1}) in 0.1 M PCA. The samples were injected into a high performance liquid chromatography (h.p.l.c.) system and NA and Ad were detected electrochemically as described previously (Livett *et al.*, 1987).

Statistics

The data were plotted as means ± s.e.mean. The amount of CA secreted in each fraction during S1 and S2 was added to obtain the total CA secretion during each stimulation. In the experiments with muscarine, the ratios of S2/S1 were calculated in order to assess the effect of SP during S2. The differences among the control and test groups were assessed by ANOVA.

Results

Effect of substance P on the time course of nicotine-evoked catecholamine secretion

The basal release of NA and Ad was in the order of 25 and 35 pmol 2 min^{-1} respectively. SP at concentrations from 10^{-10} to 10^{-5} M had no effect on basal CA release. In the absence of SP, NA and Ad secretion increased significantly during the first 6 min perfusion with 10^{-5} M nicotine and declined thereafter (Figures 1 and 2, control). NA and Ad output reached 605 ± 125 and 720 ± 100 pmol 2 min^{-1}, respectively, at maximal response. CA secretion in response to the same concentration of nicotine during S2 was reduced by approximately 50%.

SP had biphasic effects on the time course of CA secretion evoked by 10^{-5} M nicotine depending on the concentration of SP (Figures 1 and 2). In control glands perfused with nicotine (10^{-5} M), CA secretion reached its peak at 6 to 8 min and then had declined by 10 min after the start of the perfusion with

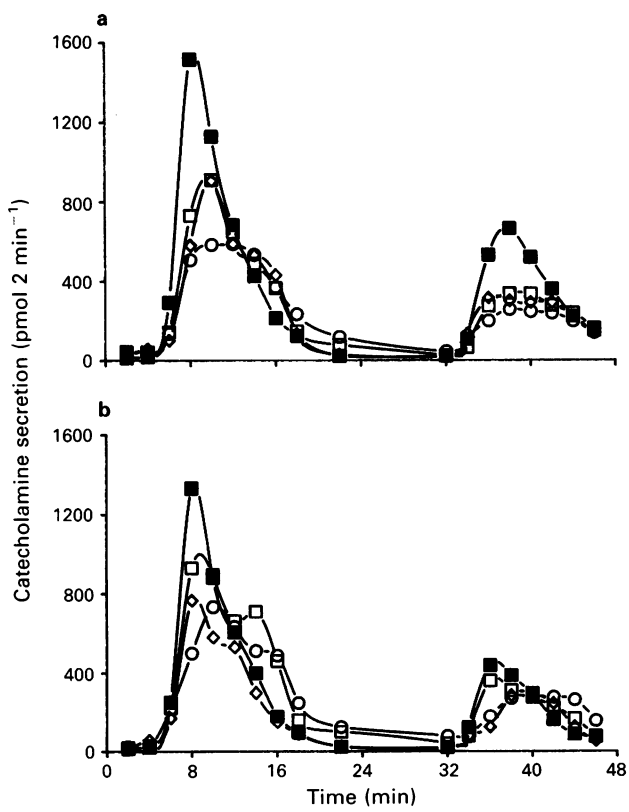

Figure 1 Effect of substance P (SP, 0, 10^{-10}, 10^{-9} or 10^{-8} M) on the time course of catecholamine (CA) secretion evoked by 10^{-5} M nicotine from rat perfused adrenal glands. After two basal samples were collected, the glands were stimulated with 10^{-5} M nicotine for 10 min twice with an 18 min interval between the two stimulations. Different concentrations of SP were added to the perfusion 2 min before and 10 during the first stimulation. Fractions were collected for CA assay every 2 min. Data are plotted as the means of 5 experiments (the error bars were 5 to 15% of the means and were omitted for clarity): (a) noradrenaline; (b) adrenaline; (○) control; (◇) SP 10^{-10} M; (■) SP 10^{-9} M; (□) SP 10^{-8} M.

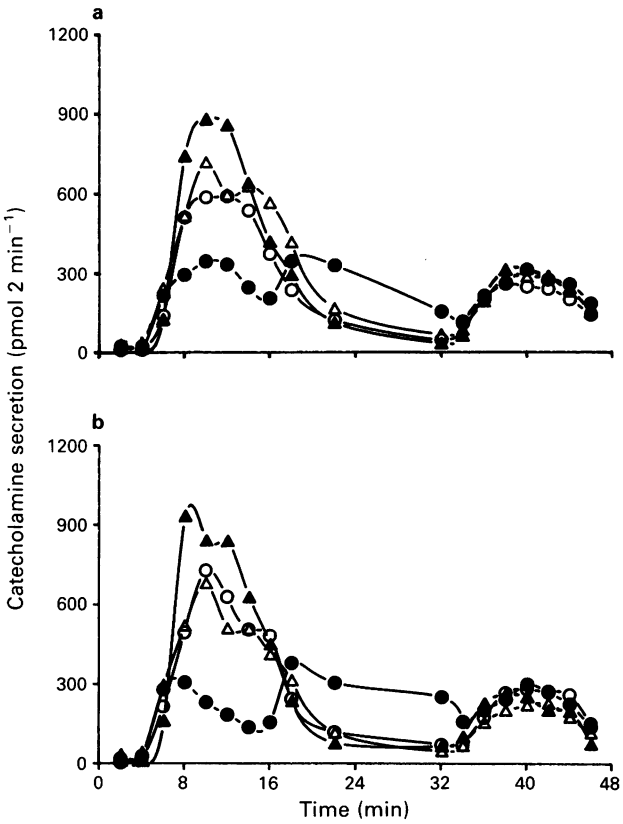

Figure 2 Effect of substance P (SP, 0, 10^{-7}, 10^{-6}, or 10^{-5} M) on the time course of catecholamine secretion evoked by 10^{-5} M nicotine from rat perfused adrenal glands. The experimental procedure was the same as in Figure 1. (a) Noradrenaline; (b) adrenaline; (○) control; (▲) SP 10^{-7} M; (△) SP 10^{-6} M; (●) SP 10^{-5} M.

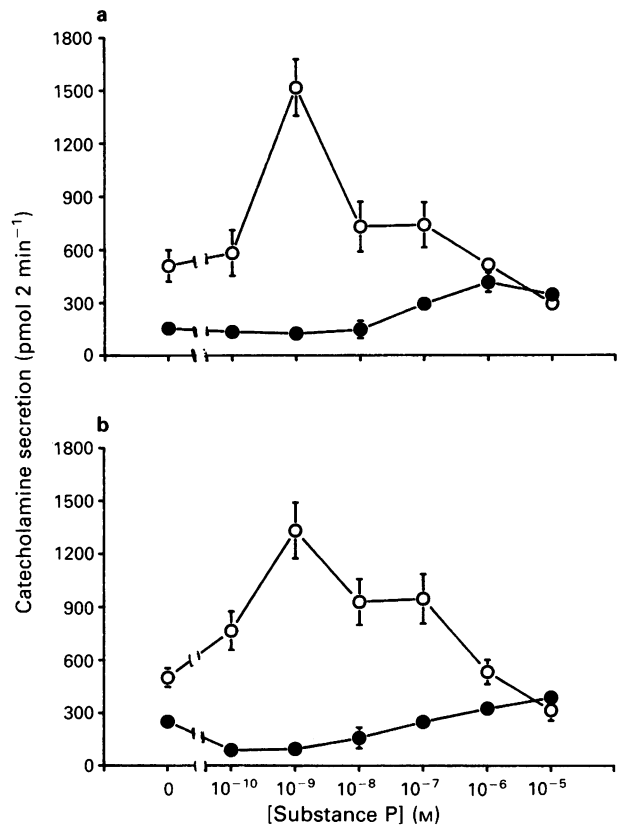

Figure 3 Effect of substance P (SP, 10^{-10}–10^{-5} M) on catecholamine (CA) secretion at 4 min and 14 min after perfusion of rat adrenal glands with nicotine (10^{-5} M). Data on CA secretion at 4 min and 14 min after nicotine in Figures 1 and 2 were replotted (means ± s.e.mean, $n = 5$) as a function of SP concentration (ANOVA test: at 4 min after nicotine, noradrenaline (NA): $F = 5.6$, $P < 0.01$; adrenaline (Ad): $F = 5.2$, $P < 0.01$; at 14 min after nicotine: NA: $F = 4.9$, $P < 0.01$; Ad: $F = 4.5$, $P < 0.01$). (a) NA; (b) Ad; (○) 4 min after nicotine; (●) 14 min after nicotine.

nicotine. In the presence of SP (10^{-9} M), CA secretion reached the maximum more rapidly, at 4 min after nicotine, when NA levels were 2.8 times and Ad levels 2.5 times higher than those in control (analysis of variance in all groups: NA: $F = 5.6$, $P < 0.01$; Ad: 4.8, $P < 0.01$) (Figure 3) and then declined rapidly by 6 and 8 min. In the presence of SP at 10^{-7}, 10^{-8} and 10^{-10} M, the changes were similar to those at 10^{-9} M but less pronounced (Figures 1, 2 and 3).

SP at 10^{-5} M also markedly changed the time course of CA secretion (Figure 2) but in a different manner from that seen with the low concentrations of SP. CA secretion in the presence of 10^{-5} M SP was almost the same as in the control during the first 2 min of nicotine stimulation; however, it decreased during the second and the third 2 min fractions. When the perfusion of SP and nicotine was stopped, NA secretion rebounded from 220 to 390 pmol 2 min^{-1} ($n = 5$) and Ad from 180 to 420 pmol 2 min^{-1} ($n = 5$). As shown in Figure 2, SP at 10^{-6} M also increased CA secretion after perfusion of SP was stopped (Figure 3).

Effect of substance P on the desensitization of the nicotinic response

In the control group (no SP in S1), the total NA and Ad output during S2 were 1440 ± 280 and 1880 ± 400 pmol (Figures 1 and 2). SP (10^{-10}–10^{-5} M) in the first 10 min stimulation had no significant effect on CA secretion during the second 10 min stimulation (in the absence of SP), 18 min later.

Effect of pre-exposure to substance P on catecholamine secretion

Pre-exposure of the adrenal glands to SP had a marked effect on the subsequent CA secretory response to nicotine. As shown in Figure 4, when SP (10^{-9} to 10^{-5} M) was present in the perfusion solution for 10 min before and 10 min during 10^{-5} M nicotine stimulation, both NA and Ad secretion were markedly inhibited in a concentration-dependent manner. As shown in Figure 4, CA secretion was almost completely inhibited by SP at 10^{-5} M. These results contrast with those where SP was exposed to the adrenal glands for only 2 min before nicotine (see Figures 1 and 2).

Effect of substance P on the time course of catecholamine secretion evoked by muscarine

As shown in Figure 5 and Figure 6, the basal secretion of NA and Ad was similar in the presence or absence of SP. In the absence of SP during S1, muscarine (10^{-4} M) preferentially increased Ad secretion (Ad/NA = 8.7 ± 0.4). In the first 2 min of stimulation during S1, NA secretion increased 2–3 fold over the basal (45 ± 5 pmol 2 min^{-1}), whereas Ad increased 12 fold over the basal to 270 ± 30 pmol 2 min^{-1}. Ad secretion continued to increase in the subsequent fractions reaching a maximum of 480 ± 80 pmol 2 min^{-1} and was maintained at this level until perfusion of muscarine was stopped. The time course and extent of Ad and NA secretion during S2 in the control was similar to that during S1. SP (10^{-9} to 10^{-5} M) for 2 min before and 10 min during S2 had no significant effect on the time course of NA or Ad secretion in S2 induced by 10^{-4} M muscarine (Figures 5 and 6).

Effect of substance P on total catecholamine secretion evoked by muscarine

In the control group, the total amount of Ad secretion evoked by 10^{-4} M muscarine over the 10 min S1 and S2 stimulation

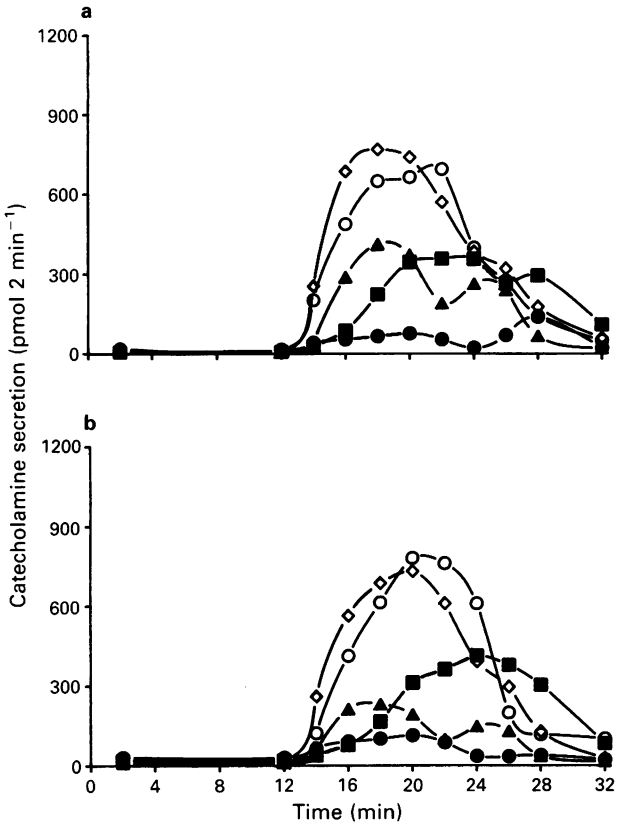

Figure 4 Effect of pre-incubation with substance P (SP) for 10 min on nicotine-induced catecholamine (CA) secretion from rat perfused adrenal glands. After a basal sample was collected, adrenal glands were perfused with SP (10^{-10} to 10^{-5} M) for 10 min before and 10 min during stimulation with 10^{-5} M nicotine. The fractions were assayed for CA and the data plotted as means of 4 experiments (error bars were 5 to 15% of means and omitted for clarity). (a) Noradrenaline; (b) adrenaline; (○) control; (◇) SP 10^{-10} M; (■) SP 10^{-9} M; (▲) SP 10^{-7} M; (●) SP 10^{-5} M.

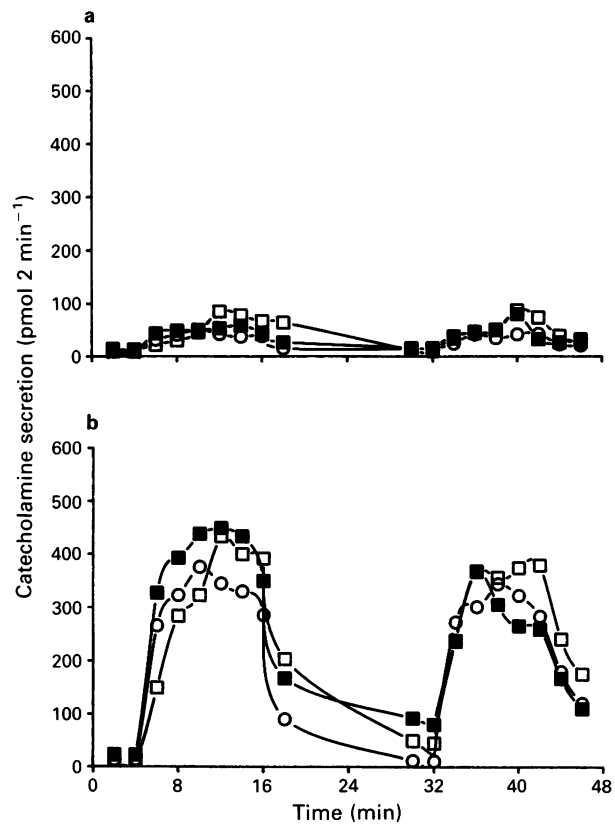

Figure 5 Effect of substance P (SP, 10^{-9}–10^{-7} M) on the time course of catecholamine (CA) secretion evoked by muscarine from rat perfused adrenal glands. After two samples were collected, the adrenal glands were perfused with 10^{-4} M muscarine for 10 min twice, with an 18 min period between. SP at concentrations from 10^{-9} to 10^{-7} M was present in the perfusion solution for 2 min before and 10 min during the second stimulation. The fractions were collected as shown in the figure for CA assay. Data are plotted as means of 5 experiments (error bars were 10%–15% of means and are omitted for the clarity). (a) Noradrenaline; (b) adrenaline; (○) control; (■) SP 10^{-9} M; (□) SP 10^{-8} M.

periods was 2012 ± 331 and 1767 ± 234 pmol, respectively and NA was 281 ± 63 and 254 ± 52 pmol respectively. In the control group (no SP), the ratios of S2/S1 were 0.95 ± 0.13 for NA and 0.84 ± 0.11 for Ad. Although the ratios of S2/S1 for NA and Ad increased slightly in the presence of SP 10^{-8} and 10^{-7} M, there were no statistically significant differences among control and test groups (ANOVA, $F = 1.17$ for Ad and $F = 0.98$ for NA, $P > 0.05$, $n = 5$).

Discussion

Facilitatory effect of substance P on the initial nicotinic response

In the present study, we compared the effects of SP on CA secretion evoked by nicotine and muscarine from perfused rat adrenal glands. We found that SP modulates nicotinic but not muscarinic CA secretion. A novel finding in the present study was that SP at the lower concentrations (10^{-10}–10^{-7} M) increased initial nicotinic CA secretion in the first two 2 min collection periods but decreased nicotinic CA secretion thereafter (Figures 1 and 2). This effect of SP was concentration-dependent, and more pronounced on NA secretion than on Ad secretion. The maximal effect occurred at 10^{-9} M SP. SP by itself had no effect on basal CA secretion from the rat adrenal glands, indicating that SP produced its effect by modulating the nicotinic secretion of adrenal catecholamines.

These results in the rat appear to differ from those in isolated bovine chromaffin cells where SP at these lower concentrations (10^{-9}–10^{-7} M) had no apparent effect on CA secretion evoked by nicotine over a 5 min collection period (Khalil *et al.*, 1988a; Boksa & Livett, 1984b). However, in the studies on adrenal chromaffin cells, an initial facilitatory effect of SP may have occurred at 2 min or 4 min but have been obscured by the fact that the release sample was collected over a 5 min-incubation period. This is reasonable, given that there are no diffusion barriers in the isolated chromaffin cells and the response to nicotine and SP could be faster than in the perfused rat preparation. Note that at 8 min after nicotine stimulation in the perfused rat preparation there was likewise no significant effect of SP (10^{-9}–10^{-7} M) on nicotine-evoked CA secretion whereas at 14 min the effects were inhibitory (Figure 3).

The mechanism by which SP facilitates CA secretion by SP in the first 4 min is not clear. SP may increase CA secretion by changing the conductance of ion channels. It has been reported that SP at 10^{-7} M depolarizes sympathetic neurones in the guinea-pig by increasing sodium permeability and decreasing potassium permeability (Dun & Minota, 1981). In autonomic ganglia, SP ($10^{-7} - 4 \times 10^{-6}$ M) mimics the non-cholinergic slow exitatory postsynaptic potential (e.p.s.p.) (Jiang *et al.*, 1982; Konishi *et al.*, 1989) and facilitates the nicotinic response (Jiang & Dun, 1986). Moreover, it has also been shown that SP at 10^{-7} M depolarizes rat chromaffin cells from the adrenal medulla by approximately 20 mV (Richter & Grunwald, 1987). Although this degree of depolarization is not sufficient to evoke CA secretion itself, the calcium influx resulting from the depolarization is thought to facilitate CA secretion evoked by nicotinic agonists.

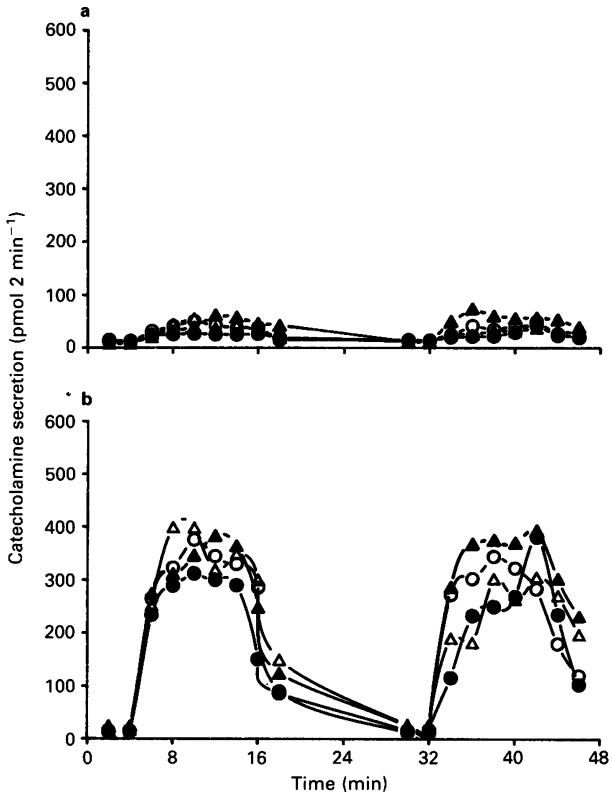

Figure 6 Effect of substance P (SP, 10^{-6}–10^{-5} M) on the time course of catecholamine secretion evoked by muscarine from rat perfused adrenal glands. The protocol was the same as in Figure 5. (a) Noradrenaline; (b) adrenaline; (○) control; (▲) SP 10^{-7} M; (△); SP 10^{-6} M; (●) SP 10^{-5} M.

It is possible that SP plays a facilitatory role in the early secretory phase via second messenger systems. It is well known that SP can stimulate hydrolysis of phospholipids and produce accumulation of inositol trisphosphate and diacylglycerol in many tissues including guinea-pig ileum, rat hypothalamus (Watson & Downes, 1983), hamster urinary bladder (Bristow *et al.*, 1987), rat parotid gland (Rollandy *et al.*, 1989) and rat skin (Thomas *et al.*, 1989). Although SP has no effect on metabolism of phosphatidylinositol phosphates (PIP) in bovine chromaffin cells (Bunn *et al.*, 1990) it does have a stimulating effect on PIP metabolism in rat adrenal medulla (Minenko & Oehme, 1987; Minenko *et al.*, 1988). An increase in cytosolic mobilization of calcium by inositol 1,4,5-trisphosphate and activation of protein kinase C by diacylglycerol may prime the calcium-sensitive secretory process so as to facilitate secretion by nicotine (Cheek *et al.*, 1989a,b). In addition, SP may facilitate nicotinic CA secretion by protecting against nicotinic receptor desensitization (Boksa & Livett, 1984b) since even low concentrations of nicotinic agonists (10^{-6} M) can cause desensitization (Boyd, 1987).

Inhibitory effect of substance P on the later nicotinic response

In the present study, we found that SP inhibited adrenal CA secretion in the later phase of nicotinic stimulation (Figures 1 and 2). This inhibitory action of SP was markedly enhanced by pre-exposure to SP for 10 min before nicotine (Figure 4). Inhibitory effects of SP on the nicotinic responses have been described for a number of different preparations including cat spinal Renshaw cells (Belcher & Ryall, 1977; Krnjevic & Lekic, 1977), bovine isolated chromaffin cells (Livett *et al.*, 1979; Role *et al.*, 1981; Boksa & Livett, 1984b), frog sympathetic ganglia and skeletal muscle endplates (Akasu *et al.*, 1983), PC-12 cells (Stallcup & Patrick, 1980; Simasko *et al.*, 1985; Boyd & Leeman, 1987), BC_3H_1 cells (Simasko *et al.*, 1985) and rat adrenal gland slices (Nieber & Oehme, 1987). This action which is Ca^{2+}-dependent and specific to nicotinic receptor function (Boksa & Livett, 1984b; Role *et al.*, 1981) has an IC_{50} for SP of about 10^{-6} M (Boksa & Livett, 1984b; Simasko *et al.*, 1987).

The mechanism by which SP inhibits nicotinic function is not clear. SP does not appear to inhibit nicotinic function by binding to the nicotine binding sites (Stallcup & Patrick, 1980; Boyd & Leeman, 1987), but rather by blocking the receptor-linked open ion channels (SP may be physically trapped within these ion channels; Stallcup & Patrick, 1980; Clapham & Neher, 1984; Simasko *et al.*, 1987). In isolated chromaffin cells and PC12 cells this action is reversible after washing out the SP (Boksa & Livett, 1984b; Boyd & Leeman, 1987). Consistent with the hypothesis that SP blocks nicotinic receptor-linked open ion channels, in the present study with the rat perfused adrenal gland preparation, CA secretion rebounded immediately after switching off both SP and nicotine. In addition, SP may act as an inhibitor of adrenal CA secretion by enhancing desensitization, as evidenced by measurement of sodium influx in rat PC-12 cells or ion currents in bovine cultured chromaffin cells (Clapham & Neher, 1984; Boyd & Leeman, 1987; Higgins & Berg, 1989).

Effect of substance P on the desensitization of the nicotinic response

In the present study, CA secretion in S2 amounted to less than 50% of S1. This reduction in CA secretion was not due to agonist-induced depletion of CA from the adrenal glands since normal adrenal glands contained 65 nmol of NA and Ad, some 20 times that secreted during S1 (Zhou & Livett, 1990a). The decline in CA secretion in S2 is more likely to be due to desensitization of the nicotinic response. SP present in S1 did not protect against the desensitization of the nicotinic response during S2. These results contrast with those in bovine cultured chromaffin cells in which SP (10^{-6} M) present together with a high concentration of nicotine (10^{-3} M) in the first incubation period protected the nicotinic response against desensitization in a second nicotinic stimulation period (Boksa & Livett, 1984b). The reason for this difference in response to SP is most likely related to the temporal differences in the experimental protocols and the previous history of the preparation (Oehme & Krivoy, 1983). The time interval between the two stimulations in the present study was longer than that in the studies in cell culture (18 min vs 20 s). Boksa & Livett (1984b) showed that the protection by SP was reversible by washout of SP. In the present study, any protective actions of SP would be lost during the prolonged perfusion time between the two stimulations.

Reports on the effect of SP on desensitization of the nicotinic response are controversial, some studies reporting SP protects against desensitization (Boksa & Livett, 1984b; Khalil *et al.*, 1988a) while others find it enhances the rate of nicotinic desensitization (Stallcup & Patrick, 1980; Clapham & Neher, 1984; Simasko *et al.*, 1985; 1987; Higgins & Berg, 1988). This apparent discrepancy of SP action may be explained by multiple actions of SP on nicotinic ACh receptors, dependent upon the time frame of these experiments and time of exposure to SP. Desensitization of the nicotinic response is known to have two phases: an initial fast phase lasting less than a second or a few seconds and a later slow phase lasting minutes or longer (Boyd, 1987). Boyd & Leeman (1987) showed that SP increased agonist-induced desensitization of nicotinic receptors in the fast phase but inhibited agonist-mediated desensitization in the later slow phase. In addition, SP also protected the receptors from agonist-mediated irreversible deactivation (Boyd & Leeman, 1987), a mechanism which may explain our finding that SP protects against nicotinic desensitization (Boksa & Livett, 1984b).

Effect of pre-exposure to substance P on the nicotinic response

The pre-exposure time with SP is important for its inhibitory action on catecholamine (CA) secretion. Boyd & Leeman (1987) reported that exposure of PC-12 cells to SP alone for 10 min caused acetylcholine receptors to become non-responsive to agonists. In the absence of nicotinic agonists SP converted the nicotinic receptor from its resting closed state to a non-functional state (Boyd & Leeman, 1987). This receptor transition did not involve the open channel state of the receptor since SP did not cause an increase in ^{22}Na influx. Our present study shows that pre-exposure of adrenal glands to SP (10^{-9} to 10^{-5} M) for 10 min caused more marked inhibition in CA secretion than did pre-exposure to SP for 2 min. In accord with the results of Boyd & Leeman (1987), this action of SP is reversible because CA secretion rebounded after termination of perfusion with SP and nicotine. This effect appears to be related to the closed state of ion channels to which SP may bind. Recently, it has been shown that bovine adrenal medullary membranes possess a novel class of binding sites for SP which unlike the NK_1 class, require both the C- and N-terminal amino acid sequence of SP (Geraghty *et al.*, 1990). The possibility that these sites represent binding of SP to the nicotinic receptor ion channel remains to be determined.

Effect of substance P on muscarinic catecholamine secretion

Muscarine at concentrations from 10^{-6} to 10^{-3} M evoked a dose-dependent secretion of Ad and NA from the perfused rat adrenal glands (data not shown). As shown in Figure 5 and Figure 6, muscarine at 10^{-4} M (close to the EC_{50}) evoked predominantly Ad secretion and had little effect on NA secretion. The Ad/NA ratio evoked by muscarine was 8.7. This contrasts with the secretion evoked by nicotine in which Ad and NA secretion were almost equal. The results in the present study in the perfused rat adrenal gland are consistent with those in the perfused cat adrenal gland described by Douglas & Poisner (1965) and Critchley *et al.* (1986). This selective secretion of Ad evoked by muscarinic agonists seems to be species-specific and was more significant in rat (Ad/NA, 8.7, present study) than in cat (Ad/NA, 3.6) and dog (Ad/NA, 2.6, Critchley *et al.*, 1986).

As shown in Figures 5 and 6, CA secretion evoked by muscarine was well maintained during the second 10 min stimulation. In contrast to nicotinic CA secretion (Figures 1 and 2), there was little desensitization of the CA secretion evoked by muscarine. SP at concentrations from 10^{-9} to 10^{-5} M had no significant effect on either the time course of the total amount of CA secretion evoked by muscarine. This result is consistent with that obtained with guinea-pig isolated chromaffin cells (Role *et al.*, 1981) and in other systems (Krnjevic & Lekic, 1977; Belcher & Ryall, 1977). The reason for the differential effects of SP on nicotinic and muscarinic CA secretion may reflect the different pathways for secretion activated by nicotine and muscarine (Harish *et al.*, 1987; Cheek *et al.*, 1989a,b).

In conclusion, SP has multiple actions on nicotinic CA secretion from the perfused rat adrenal gland *in vitro*. SP at low concentrations (10^{-7}–10^{-9} M) changed the time course of CA secretion resulting in enhancement of CA secretion in response to nicotine in the first 4 min and inhibition of secretion thereafter. At higher concentrations (10^{-5} M), SP inhibited total CA secretion followed by a rebound in CA secretion after wash out of the SP. Pre-exposure of adrenal glands to SP (10^{-9} to 10^{-5} M) for 10 min produced a more profound inhibition in CA secretion than pre-exposure to SP for 2 min. SP had no effect on muscarinic CA secretion. These results with the rat adrenal gland perfused *in vitro* suggest both a quantitative and temporal role for SP as a novel modulator of adrenal CA secretion.

We thank Mr Ian Young, Mr Tim Anning and Mr Phillip McGlashan for technical assistance. X.-F.Z. was supported by a University of Melbourne Postgraduate Award. P.D.M. is an NH&MR Research Fellow. This work was supported by a project grant to B.G.L. from the National Health and Medical Research Council (NH&MRC) of Australia.

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(Received June 6, 1990
Revised October 22, 1990
Accepted November 22, 1990)