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INHIBITORY SYNAPTIC POTENTIALS RECORDED FROM MAMMALIAN NEURONES PROLONGED BY BLOCKADE OF NORADRENALINE UPTAKE

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SUMMARY

1. Intracellular recordings of membrane potential and membrane current were made from neurones of the rat nucleus locus coeruleus and the guinea-pig submucous plexus. These neurones exhibit inhibitory post-synaptic potentials (i.p.s.p.s) which result from noradrenaline acting on α_2-adrenoceptors to cause an increase in potassium conductance.

2. Cocaine (0·2–30 μM) reversibly increased the duration of the i.p.s.p. or inhibitory post-synaptic current (i.p.s.c.) in locus coeruleus neurones and submucous plexus neurones by approximately 750% and 350% respectively. The concentrations of cocaine causing half-maximal prolongation of the synaptic current were 3 μM in locus coeruleus and 0·5 μM in submucous plexus. The prolongation was due entirely to a slower rate of decay of the synaptic response.

3. Cocaine (10 μM) produced a maintained hyperpolarization (2–10 mV) or outward current (20–120 pA) in locus coeruleus neurones; in submucous plexus neurones cocaine increased the amplitude and duration of spontaneous i.p.s.p.s.

4. Outward currents produced by superfusion with noradrenaline were increased by cocaine with maximum effects being observed at 10–30 μM-cocaine. The maximum leftward shift in the relation between outward current or membrane hyperpolarization and noradrenaline concentration was 18- to 100-fold in locus coeruleus neurones and 4-fold in submucous plexus neurones. The concentrations of cocaine which caused a half-maximal increase in sensitivity to superfused noradrenaline were similar in both tissues, being 4 μM in locus coeruleus and 2 μM in submucous plexus.

5. These results show that neuronal uptake of noradrenaline released from adrenergic nerves plays a significant role in determining the time course of synaptic potentials mediated by noradrenaline.

INTRODUCTION

Inactivation of synaptically released neurotransmitters is accomplished by diffusion of the transmitter away from its post-synaptic receptors and by enzymatic degradation and/or reuptake. Enzymatic degradation plays a significant role in inactivating

acetylcholine at the nicotinic synapse, where anticholinesterases prolong the duration of the end-plate current at the skeletal neuromuscular junction (Magleby & Stevens, 1972; Katz & Miledi, 1973; Hartzell, Kuffler & Yoshikami, 1975) or the excitatory post-synaptic potential (e.p.s.p.) at neuronal nicotinic synapses (Dennis, Harris & Kuffler, 1971; Bornstein, 1974). In amphibian parasympathetic neurones, acetylcholine also activates muscarinic receptors to produce a long-lasting (2 s) inhibitory post-synaptic potential (i.p.s.p.); this muscarinic i.p.s.p. lasts up to 35 s in the presence of anticholinesterases (Hartzell, Kuffler, Stickgold & Yoshikami, 1977).

The actions of inhibitors of catecholamine reuptake on synaptic potentials mediated by noradrenaline are less well known although there is a large body of indirect evidence that suggests a significant proportion of the inactivation of noradrenaline released from adrenergic terminals is due to neuronal uptake mechanisms (Cooper, Bloom & Roth, 1982). Uptake of exogenously added radiolabelled catecholamines has been demonstrated in peripheral and central neurones *in vivo*, in isolated peripheral tissues as well as brain slices and synaptosomal preparations (Glowinski, Kopin & Axelrod, 1965; Iversen, 1968, 1971; Snyder & Coyle, 1969; Langer, 1970; Koe, 1976; Reith, Sershen, Allen & Lajtha, 1983). Autoradiographic studies have demonstrated that the major site of noradrenaline uptake is on the sympathetic and central noradrenergic nerve fibres themselves (Lee & Snyder, 1981; Raisman, Sette, Pimoule, Briley & Langer, 1982; Rehavi, Skolnick, Brownstein & Paul, 1982); in peripheral tissue these uptake sites disappear after sympathectomy (Raisman *et al.* 1982). Cocaine is well known to enhance the peripheral effects of post-ganglionic sympathetic stimulation (Trendelenburg, 1968; Iversen, 1968) and many of the profound behavioural effects of cocaine have been tentatively attributed to an enhancement of the actions of centrally released catecholamines (Kuczenski, 1983; Wise, 1984). However, little is known about the contribution this uptake process makes to the termination of action of noradrenaline at the level of the individual synapse.

Inhibitory synaptic potentials mediated by the release of noradrenaline have been described in neurones of the rat locus coeruleus and neurones of the guinea-pig submucous plexus (Egan, Henderson, North & Williams, 1983; North & Surprenant, 1985; Mihara, Katayama & Nishi, 1985). The release of noradrenaline onto these neurones produces i.p.s.p.s which result from an increased potassium conductance due to activation of α_2-adrenoceptors on the post-synaptic membrane (Egan *et al.* 1983; Williams, Henderson & North, 1985; Mihara *et al.* 1985; North & Surprenant, 1985). In submucous plexus neurones, noradrenaline is released from extrinsic sympathetic nerve fibres (see Furness & Costa, 1980); in locus coeruleus neurones the source of noradrenaline may be axon collaterals and/or the cell bodies or dendrites of the noradrenaline-containing locus coeruleus neurones themselves. These i.p.s.p.s provide the opportunity to measure directly the extent to which noradrenaline uptake contributes to neurotransmission at individual sympathetic and central adrenergic synapses. We previously found that an irreversible neuronal uptake inhibitor, desmethylimipramine (DMI), markedly increased the duration of the i.p.s.p. in locus coeruleus neurones but decreased the amplitude of all synaptic potentials in submucous plexus neurones (Egan *et al.* 1983; North & Surprenant, 1985). The first purpose of the present experiments was to determine whether the

reversible uptake inhibitor, cocaine, affected the i.p.s.p.s at these synapses. We found that cocaine increased the amplitude and duration of the i.p.s.p.s recorded from both locus coeruleus and submucous plexus neurones. The second purpose was to establish whether these actions were likely to result from a block of noradrenaline uptake.

METHODS

Details of the methods used to prepare tissues used in the present study have been described previously (Williams, North, Shefner, Nishi & Egan, 1984; North & Surprenant, 1985). The procedures were as follows:

Submucous plexus. Preparations of submucous plexus were obtained from the small intestine of young guinea-pigs (150–250 g). The mucosa was stripped away from segments of the small intestine; the submucous plexus sheath was pulled away from the underlying circular smooth muscle and pinned out in an organ bath (volume 0·75 ml) through which physiological saline flowed continuously at a rate of 2 ml/min. Temperature was maintained at 35–37 °C. Outlines of individual neurones within a node, or ganglion, of the plexus were visualized using Nomarski optics at ×320 magnification; each node contained from 4 to 30 neurones. Nerves were stimulated by placing a micro-electrode (tip diameter approx. 30 μm) filled with physiological saline onto one of the interganglionic nerve fibre bundles projecting into the node under investigation; current was passed between two platinum wires, one of which was inserted into the micro-electrode, the other being placed around the outside of this stimulating electrode.

Locus coeruleus. Male rats (150–200 g) were anaesthetized with ether and killed by a heavy blow to the chest. The brain was removed; a portion of the brain stem containing the pons was placed in a vibratome and 300 μm slices cut. A single slice containing the caudal part of the locus coeruleus was placed in a tissue bath (volume 0·5 ml) and was completely submerged in a flowing solution of physiological saline (1·5 ml/min) at 37 °C. The area of the locus coeruleus was easily identifiable as a relatively translucent, crescent-shaped area on the ventrolateral border of the fourth ventricle; this was readily observed under transmitted light at 10–50 × magnification. Intracellular recordings were obtained from neurones in this area; individual locus coeruleus neurones were identified on the basis of their membrane properties (Williams *et al.* 1984). Synaptic potentials were evoked by electrical stimulation from bipolar tungsten electrodes inserted into the brain slice in the area near the locus coeruleus.

The physiological saline solution used for these preparations differed only in the concentration of KCl, being 5 mM in experiments performed on submucous plexus preparations and 2·5 mM in experiments with locus coeruleus preparations. The composition was otherwise as follows (mM): NaCl, 126; NaH_2PO_4, 1·2; $MgCl_2$, 1·2; $CaCl_2$, 2·5; $NaHCO_3$, 25; glucose, 11; gassed with 95% O_2 and 5% CO_2.

Drugs were applied by changing the superfusion solution by means of three-way taps so that the flow rate did not change. Approximately 20–40 s elapsed between turning the tap to entry of the changed solution into the bath; effects of drugs reached a steady state within 30–90 s of the solution entering the bath.

Intracellular recordings were made using micro-electrodes filled with 2 M-KCl. Micro-electrodes having resistances of 30–80 MΩ were used to impale locus coeruleus neurones; electrodes with resistances of 60–100 MΩ were used for submucous plexus neurones. Membrane potentials and membrane currents were recorded using a single-electrode voltage-clamp amplifier (Axoclamp II or Dagan 8100). Voltage at the amplifier headstage was monitored on a separate oscilloscope to ensure correct operation of the switch clamp; switching frequency was 3–6 kHz (locus coeruleus neurones) or 1–3 kHz (submucous plexus neurones), duty cycle was 30 or 50%. Voltage-clamp data were accepted when potential changes were less than 1–5% of the unclamped potential changes.

The following drugs were used: (−)-noradrenaline bitartrate (Sigma); desmethylimipramine hydrochloride (DMI; Ciba-Geigy); idazoxan (Reckitt and Colman); cocaine (Sigma).

All mean values given in the text are expressed as the mean ± S.E. of mean. Tests of significance were made using Student's *t* test.

RESULTS

Effects of cocaine on i.p.s.p.s

I.p.s.p.s recorded from locus coeruleus and submucous plexus neurones were similar to those described previously (Hirst & Silinsky, 1975; Egan *et al.* 1983; North & Surprenant, 1985; Mihara *et al.* 1985). I.p.s.p.s evoked in response to a single, supramaximal stimulus, in the absence of uptake inhibitors, were approximately 1–2 s in duration (Fig. 1). Under voltage clamp, nerve stimulation evoked an

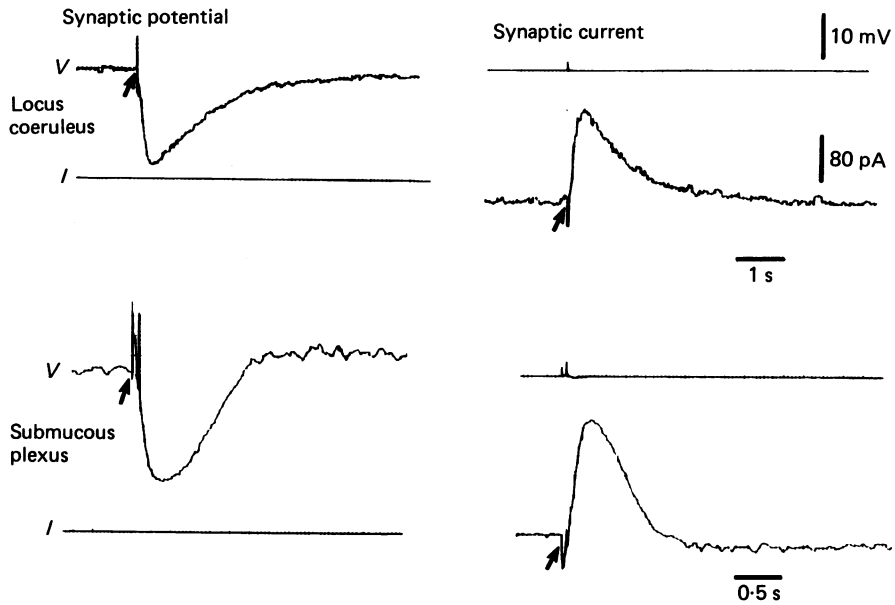

Fig. 1. Adrenergic i.p.s.p.s (left-hand traces) and i.p.s.c.s (right-hand traces) recorded from locus coeruleus and submucous plexus neurones. Locus coeruleus: a single stimulus (arrow) evoked an e.p.s.p. followed by the i.p.s.p. of 20 mV amplitude and 2 s duration. When the neurone was voltage clamped at −60 mV the same stimulus evoked an outward current (i.p.s.c.) whose time course was similar to the i.p.s.p. Submucous plexus: two stimuli (arrow) evoked the nicotinic e.p.s.p.s and an i.p.s.p. of 25 mV amplitude and 1 s duration. The neurone was then clamped at its resting potential (−55 mV); the i.p.s.c. evoked by two pulses is shown. Voltage and current calibrations apply to all traces. Note different time scales.

outward current, the inhibitory post-synaptic current (i.p.s.c.), whose time course was similar to that of the i.p.s.p. (Fig. 1). The maximum amplitude of the i.p.s.c. evoked in response to multiple stimuli (five pulses at 20 Hz in submucous neurones; five pulses at 15 Hz in locus coeruleus neurones) was 200–300 pA in both of these neuronal populations.

Cocaine (0·2–10 μM) increased the duration of the i.p.s.p. or i.p.s.c. (Fig. 2). The prolongation of the i.p.s.p. was observed within 2–4 min after introduction of cocaine into the superfusion fluid and was reversible within 20 min after wash-out of the cocaine (Fig. 2). It can be seen from the traces shown in Fig. 2 that the prolongation of the i.p.s.p. was due to a slower rate of decay of the synaptic potential; cocaine

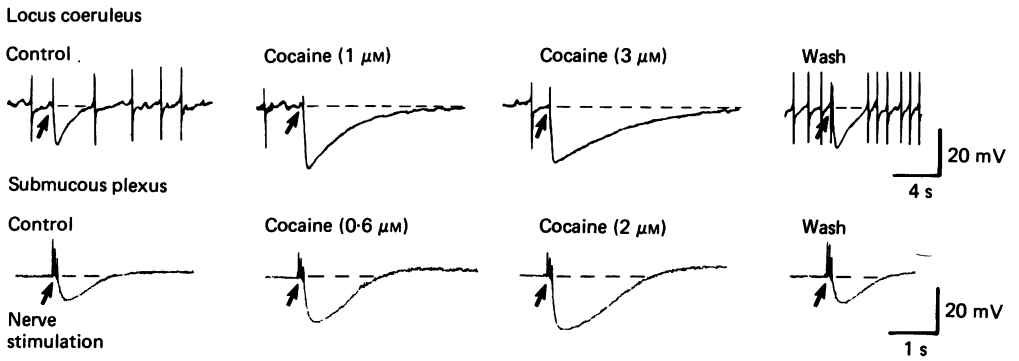

Fig. 2. Cocaine prolonged i.p.s.p.s recorded from locus coeruleus (top traces) and submucous plexus (lower traces) neurones. Top traces show the i.p.s.p. evoked in response to a single pulse stimulus (arrows) in control, in 1 and 3 μM-cocaine and about 20 min after wash-out of the cocaine. Lower traces show the i.p.s.p. evoked in response to three stimuli (arrows) at 20 Hz in control, 0·6 and 2 μM-cocaine and 20 min after returning to control solution.

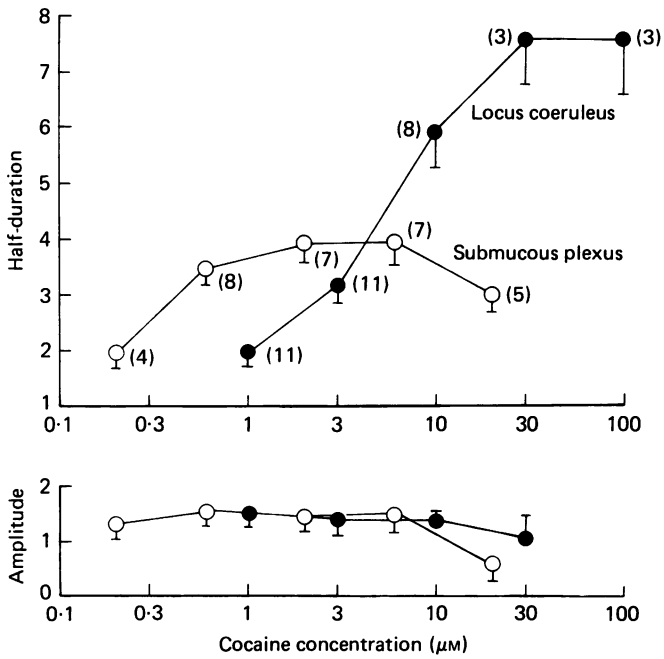

Fig. 3. Effects of cocaine on the half-duration and amplitude of i.p.s.p.s or i.p.s.c.s recorded from locus coeruleus (●) and submucous plexus (○) neurones. Results are expressed as fold increase over control, where control = 1. Numbers in parentheses are numbers of cells tested. The EC_{50} values for prolongation of the synaptic response were 0·5 μM in submucous neurones and 3 μM in locus coeruleus neurones.

had no significant effect on the rise time of the i.p.s.p. Cocaine (0·2–2 μM) also increased the amplitude of the i.p.s.p. and i.p.s.c. when their control values were less than about 15 mV and 150 pA, respectively.

The effects of cocaine on submaximal i.p.s.p.s or i.p.s.c.s evoked in response to one

or two nerve stimuli were examined in seventeen submucous plexus neurones and thirty-seven locus coeruleus neurones. In the submucous plexus neurones, the average control amplitude of the i.p.s.p. was $14 \pm 1{\cdot}2$ mV ($n = 10$) and of the i.p.s.c. 94 ± 9 pA ($n = 7$) with half-duration of 752 ± 30 ms ($n = 17$). In the locus coeruleus, these values were $14{\cdot}5 \pm 0{\cdot}9$ mV ($n = 26$), $73{\cdot}8 \pm 5$ pA ($n = 11$) and 1000 ± 40 ms ($n = 37$), respectively. The results of these experiments are shown in Fig. 3 where half-duration and amplitude of the inhibitory synaptic responses are plotted as a function of cocaine concentration. Cocaine produced a concentration-dependent prolongation of the synaptic response in both neuronal types. The maximum effect of cocaine was much greater in locus coeruleus neurones than in submucous neurones; cocaine prolonged the half-duration of the synaptic response by a maximum of about 350% in submucous neurones and 750% in locus coeruleus neurones (Fig. 3*A*). Cocaine EC_{50} values (i.e. concentration of cocaine producing half-maximal effects) for prolongation of the inhibitory synaptic response were 0·5 μM in submucous plexus and 3 μM in locus coeruleus. On the other hand, the amplitude of the synaptic response was increased by the same amount (aproximately 50%) in both locus coeruleus and submucous plexus neurones (Fig. 3*B*).

Effects of desmethylimipramine (DMI) on i.p.s.p.s

DMI (1 μM) prolonged the i.p.s.p. in locus coeruleus neurones by 600 ± 74% ($n = 12$). The prolongation of the i.p.s.p. by DMI was not reversed within 5–7 h after removing DMI from the superfusion fluid. Cocaine (10 μM) produced no further increase in the duration or amplitude of the i.p.s.p. in neurones which had been exposed to DMI ($n = 2$). In submucous plexus neurones, the i.p.s.p. was unaltered or decreased in amplitude and duration during superfusion with DMI (0·2–20 μM) for 5–20 min ($n = 12$) (see North & Surprenant, 1985). A transient increase in amplitude and duration was observed in four of twelve neurones in the first 3 min of superfusion with DMI; this was followed by a gradual decrease in the i.p.s.p. over the next 10–15 min. Upon wash-out (20–30 min) of DMI the i.p.s.p. returned to, or was slightly increased over, control values. However, cocaine (0·2–2 μM) produced no significant increase in the duration of the i.p.s.p. after exposure to 2 μM-DMI ($n = 4$).

As is apparent from Fig. 3, the amplitude (and half-duration in submucous plexus) of the synaptic response decreased when the concentration of cocaine was greater than 10 μM. One factor which may be responsible for this depression of the inhibitory synaptic response is a local anaesthetic effect on the presynaptic terminals; indeed, prolonged exposure to high (30 μM or greater) concentrations of cocaine always depressed the amplitude or rate of rise of directly evoked action potentials in the cell bodies. However, it is also possible that some of this depressant action of cocaine may be due to presynaptic inhibition of noradrenaline release caused by activation of presynaptic α_2-adrenoceptors by elevated levels of endogenous (extracellular) noradrenaline. This seems particularly likely in the case of locus coeruleus neurones because the depression of the i.p.s.p. or i.p.s.c. was observed within 2–5 min after addition of 30 μM-cocaine (which produced a maintained hyperpolarization or outward current; see below, 'Effects of cocaine on spontaneous release of noradrenaline'), while the local anaesthetic effect appeared only after prolonged exposure. Moreover, concentrations of cocaine or DMI lower than those having a local anaesthetic action still depressed the amplitude of the synaptic response.

Effects of cocaine on spontaneous release of noradrenaline

Locus coeruleus. The spontaneously occurring action potentials, which are characteristic of locus coeruleus neurones *in vitro* were always reduced in frequency when cocaine (1–100 μM) was applied to the superfusion solution; in many neurones cocaine also hyperpolarized the membrane by 2–10 mV. The reduced frequency of action

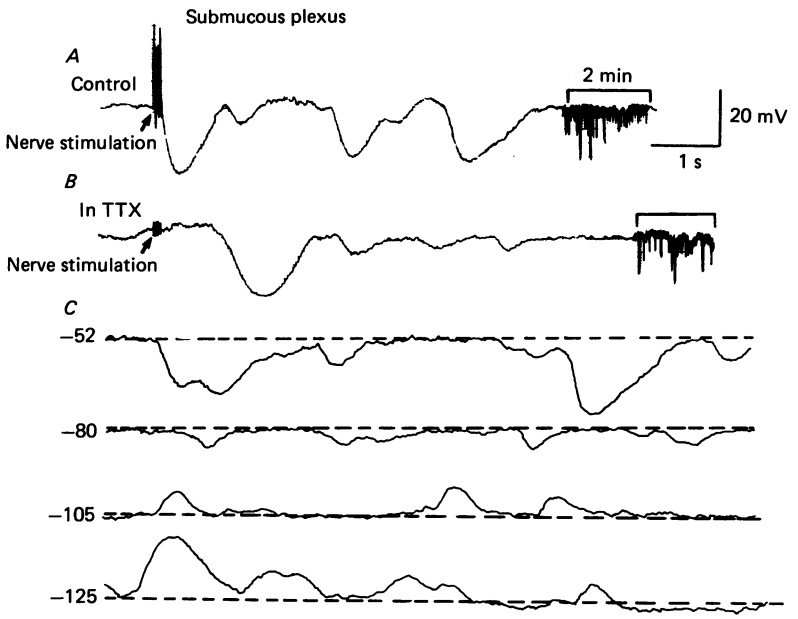

Fig. 4. Spontaneous i.p.s.p.s recorded from submucous plexus. *A*, the evoked i.p.s.p. in response to three stimuli at 20 Hz (arrow) is followed by several spontaneous hyperpolarizations having the same time course as the evoked i.p.s.p. *B*, spontaneous i.p.s.p.s were recorded in the presence of TTX (300 nM) which abolished all evoked synaptic potentials (arrow, eight pulses at 40 Hz, maximum stimulus intensity). The larger amplitude spontaneous i.p.s.p.s are apparent as downward deflexions on the slower time base shown at the end of the traces in *A* and *B*. *C*, reversal of spontaneous i.p.s.p.s. Spontaneous i.p.s.p.s recorded at resting potential (−52 mV) and when the membrane was held at −80, −105 and −125 mV by passing steady current through the recording electrode. Each segment shows the largest amplitude spontaneous potential recorded at resting potential and during a 1 min period at each of the hyperpolarized potentials. At membrane potentials more negative than −100 mV spontaneous depolarizations of similar time course to the spontaneous i.p.s.p.s were observed. The reversal potential of the evoked i.p.s.p. recorded from this neurone was −97 mV. Recordings in *C* were obtained in the presence of TTX; all recordings in *A–C* were from the same neurone.

potential firing, or the membrane hyperpolarization, occurred within 1–2 min of the cocaine reaching the tissue, was maintained during the application of cocaine and gradually returned to control levels within 20 min after returning to the normal superfusion solution. When the membrane was clamped at −60 mV (below threshold for spontaneous action potential initiation), cocaine superfusion resulted in a maintained outward current of 20–120 pA. The α_2-adrenoceptor antagonist, idazoxan (1 μM), rapidly and completely reversed the outward current produced by superfusion with cocaine. DMI (1–10 μM) produced similar effects (see Egan *et al.* 1983).

Submucous plexus. Superfusion with 0·2–2 μM-cocaine produced a 5–15 mV maintained hyperpolarization in three of thirty-five neurones examined, increased the amplitude and duration of spontaneous i.p.s.p.s in all neurones which exhibited a high frequency of spontaneous i.p.s.p.s (see below), and had no effect on the resting

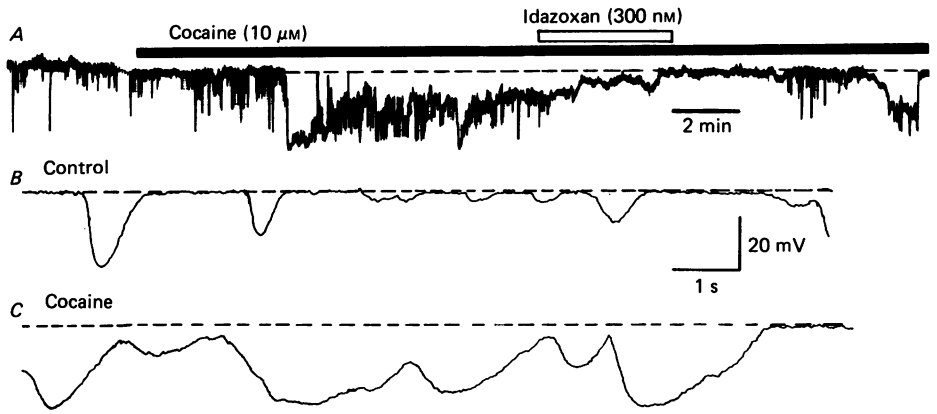

Fig. 5. Effects of cocaine on spontaneous i.p.s.p.s. *A*, voltage recording on slow time base is shown; cocaine (10 μM) was present for the period indicated by the filled bar. In control solution spontaneous i.p.s.p.s occurred at 1–4/s; the majority of these spontaneous i.p.s.p.s were less than 4 mV in amplitude and are not discernible on the slow time base but are apparent on the faster sweep speed shown in *B*. In the presence of cocaine there was an increase in the occurrence of large amplitude spontaneous i.p.s.p.s which often 'fused' (*C*) so as to produce erratic waves of hyperpolarizations. These types of spontaneous i.p.s.p.s were never observed in the absence of cocaine. The α_2-adrenoceptor antagonist, idazoxan (300 nM; open bar) abolished all spontaneous i.p.s.p.s.

membrane properties of the remaining neurones. Spontaneous membrane hyperpolarizations of 2–25 mV amplitude and 0·5–1 s duration were often observed during intracellular recordings from submucous neurones (see Surprenant, 1984); in the majority of impalements their frequency was much less than 1 min. However, in eight cells these transient spontaneous hyperpolarizations occurred sufficiently often to permit further study. Fig. 4 shows recordings of evoked i.p.s.p.s and the spontaneous hyperpolarizations obtained in one of these neurones; it can be seen that the evoked and spontaneous potentials were quite similar. When the membrane was held at potentials more negative than resting potential, by applying steady inward current through the micro-electrode, the spontaneous hyperpolarizations were reduced in amplitude and, at potentials more negative than −95 to −100 mV, spontaneous depolarizations of similar time course were recorded (Fig. 4*C*). Idazoxan (300 nM) completely abolished these spontaneous potentials within 2 min after it was added to the bathing fluid and the spontaneous hyperpolarizations returned within 10 min of wash-out of idazoxan (Fig. 5). These results provide good evidence that the spontaneous hyperpolarizations are due to the release of noradrenaline from the sympathetic terminals; that is, they are spontaneous inhibitory synaptic potentials. In the present study tetrodotoxin (TTX, 300 nM) reduced or abolished the spontaneous i.p.s.p.s in only two of the eight neurones examined but had no apparent

effect on the frequency or amplitude of the spontaneous i.p.s.p.s recorded from the other neurones (Fig. 4*B* and *C*); TTX always abolished all evoked synaptic potentials. These results indicate that the spontaneous i.p.s.p.s can result from spontaneous action potential activity in the sympathetic fibres or spontaneous release of noradrenaline from the sympathetic terminals.

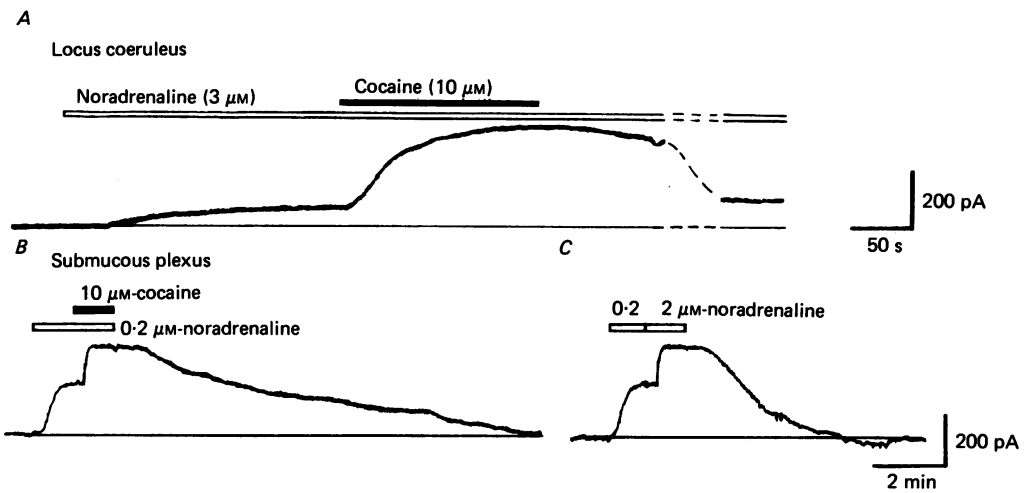

Fig. 6. Cocaine increases the outward current produced by superfusion with noradrenaline in locus coeruleus (*A*) and submucous plexus neurones (*B*). *A*, voltage-clamp recording of membrane current obtained from a locus coeruleus neurone; superfusion with 3 μM-noradrenaline (open bar) evoked an outward current of 60 pA. When 10 μM-cocaine was added to the superfusion fluid (filled bar), the outward current increased to 300 pA. Interrupted lines indicate a period of approximately 20 min during which cocaine (but not noradrenaline) was washed out. *B*, a similar voltage-clamp experiment performed on a submucous plexus neurone. Noradrenaline (200 nM) caused an outward current which was increased by 10 μM-cocaine. *C*, outward current produced by superfusion with 200 nM and then 2 μM-noradrenaline in the absence of cocaine; maximum outward current was 330 pA. Recordings of *B* and *C* were obtained from the same neurone. Note faster recovery in *C*. Note difference in time scales between recording obtained in locus coeruleus and submucous plexus neurones. Holding potentials = −55 mV.

The voltage recording shown in Fig. 5 illustrates the enhancement of occurrence of large amplitude spontaneous i.p.s.p.s produced by superfusion with cocaine. It can be seen that cocaine did not simply hyperpolarize the membrane; rather cocaine increased the amplitude and duration of spontaneous i.p.s.p.s such that individual spontaneous i.p.s.p.s merged one into the other so as to produce irregular, long-lasting 'waves' of hyperpolarizations (Fig. 5*A* and *C*).

Effects of cocaine on responses to exogenous noradrenaline

Noradrenaline, when added to the superfusion solution for periods of 2–30 min, produces maintained, dose-dependent membrane hyperpolarizations in neurones of the locus coeruleus and submucous plexus (Williams *et al.* 1985; North & Surprenant, 1985). Under voltage-clamp conditions, noradrenaline produced maintained outward currents in these neurones (Fig. 6); the threshold concentration required to evoke an

outward current was approximately 10 μM in locus coeruleus neurones and 20–60 nM in submucous neurones. The maximum amplitude of the outward current recorded from submucous neurones ranged from 200 to 350 pA ($n = 8$) and was observed at noradrenaline concentrations of 0·6–6 μM (Fig. 6*C*), while the amplitude of the outward current produced by 100 μM-noradrenaline (the highest concentration

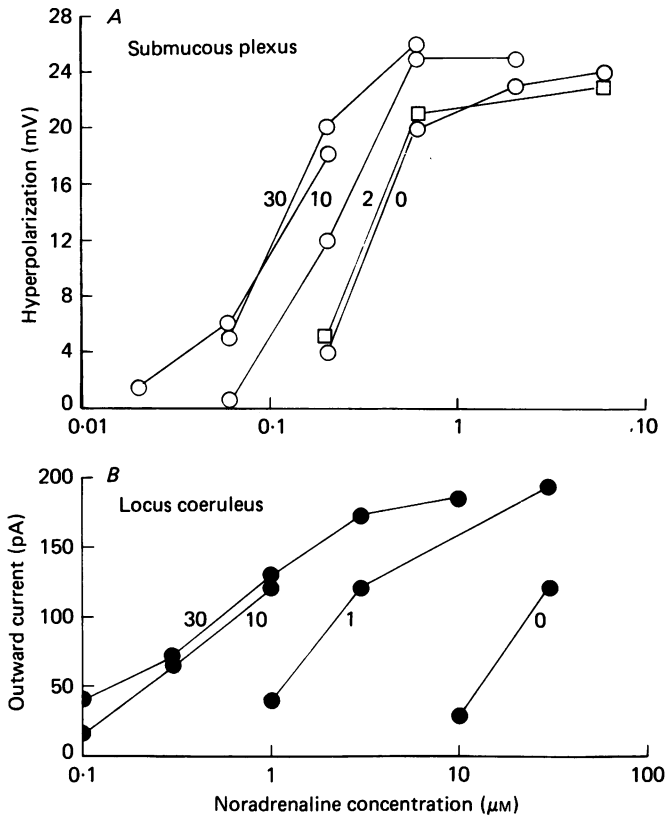

Fig. 7. Noradrenaline concentration–response curves obtained from one submucous plexus neurone (*A*) and one locus coeruleus neurone (*B*) in the absence and then presence of increasing concentrations of cocaine (as indicated in μM by each curve). *A*, hyperpolarizations in response to 2 min superfusions with noradrenaline are shown; open squares represent hyperpolarizations recorded approximately 40 min after wash-out of all cocaine. *B*, outward currents in response to noradrenaline superfusion. Cocaine shifted the concentration–response curves to the left in an approximately parallel fashion.

tested) ranged from 75 to 255 pA in locus coeruleus neurones ($n = 12$). When the superfusion solution was changed from one which contained noradrenaline to one containing both noradrenaline and cocaine, the outward current increased rapidly. Examples from experiments on locus coeruleus and submucous neurones are shown in Fig. 6*A* and *B*. Fig. 6*A* shows the membrane current recorded from a locus coeruleus neurone; superfusion with 3 μM-noradrenaline produced a steady outward current of 60 pA which rose to a maintained outward current of 310 pA within 50 s of adding cocaine (10 μM) into the noradrenaline-containing superfusion fluid. A

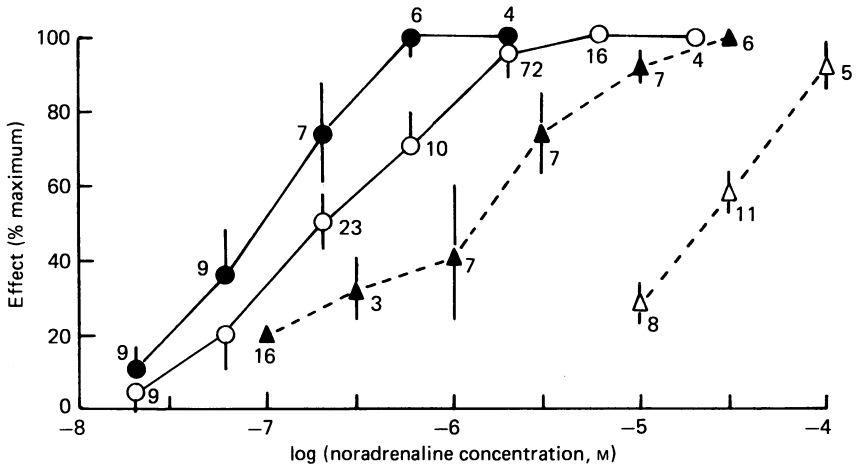

Fig. 8. Concentration–response curves obtained from all experiments performed on locus coeruleus (△, ▲) and submucous plexus neurones (○, ●) in control solution (△, ○) and in the presence of a maximum concentration of cocaine (30 or 100 μM; ▲, ●). Response measured was outward current in all experiments on locus coeruleus neurones, hyperpolarization or outward current in experiments on submucous plexus neurones. Numbers beside each point refer to total number of cells; each point is the mean ± S.E. of mean.

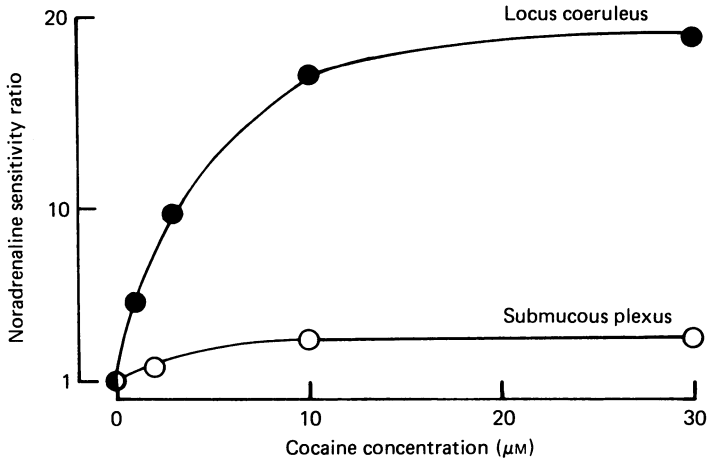

Fig. 9. Change in noradrenaline sensitivity caused by cocaine in locus coeruleus (●) and submucous plexus (○) neurones. The noradrenaline sensitivity ratios are plotted as a function of the cocaine concentration. Maximum sensitivity ratios obtained in the experiments shown were 17·5 in the locus coeruleus and 3·8 in the submucous plexus. The concentrations of cocaine which produced half-maximal effects (EC_{50} values) in these two experiments were 3·3 and 1·9 μM respectively.

voltage-clamp recording obtained from one submucous neurone is shown in Fig. 6*B* where it can be seen that the outward current of 180 pA which resulted from superfusion with 200 nM-noradrenaline was increased to 330 pA by the addition of 10 μM-cocaine. The effects of cocaine were reversible within 10–20 min after removing cocaine from the superfusion solution (Fig. 6*A* and *B*).

The ability of cocaine to enhance the actions of noradrenaline on these neurones

was examined in more detail by constructing concentration–current (or hyperpolarization) curves in the absence and presence of several concentrations of cocaine. Results obtained from one such experiment on a locus coeruleus neurone are shown in Fig. 7*B* where the amplitude of the outward current is plotted as a function of the noradrenaline concentration. Cocaine shifted the noradrenaline dose–response curve to the left with maximum effects being observed at cocaine concentrations of 30 μM. Concentration–current curves were obtained in four other locus coeruleus neurones to which at least three concentrations of cocaine (1–100 μM) were applied; in these neurones cocaine also produced leftward shifts in the dose–response curves with maximum shifts being observed at 10–30 μM-cocaine. Similar experiments were carried out on four submucous plexus neurones; in one of these neurones the response recorded was outward current while in the other three neurones hyperpolarization in response to noradrenaline was measured (Fig. 7*A*). Here too, cocaine produced a dose-dependent leftward shift of the noradrenaline concentration–response curve with the maximum shift being obtained at 10–30 μM-cocaine.

Results from these experiments were then used to calculate dose ratios, or 'noradrenaline sensitivity ratios' (Kenakin, 1980, 1984) by dividing the concentration of noradrenaline required to produce a given response (i.e. 50% of maximum) in the absence of cocaine by the concentration of noradrenaline causing the same response in the presence of cocaine. The maximum noradrenaline sensitivity ratio produced by cocaine showed considerable variation among locus coeruleus neurones, ranging from 14·5 to 118 (average $43{\cdot}7 \pm 19$; $n = 5$). On the other hand, maximum noradrenaline sensitivity ratios obtained in submucous neurones showed remarkably little variation, being $3{\cdot}6 \pm 0{\cdot}3$ ($n = 4$); these values are an order of magnitude less than those obtained in the locus coeruleus. Fig. 8 summarizes the results obtained from all experiments performed on locus coeruleus and submucous plexus neurones in which the effects of maximum concentrations of cocaine (10 or 30 μM) were examined.

The noradrenaline sensitivity ratio determined at each concentration of cocaine examined was plotted as a function of the cocaine concentration; data obtained from single experiments are plotted in Fig. 9 where it can be seen that maximum shifts were obtained in each case. These findings indicate that the higher concentrations of cocaine used in the present study were, indeed, saturating the noradrenaline uptake sites in these neurones. This allowed us to determine EC_{50} values for cocaine; i.e. the concentration of cocaine which caused a half-maximal shift in the noradrenaline dose ratio. Cocaine EC_{50} values were $4 \pm 0{\cdot}75$ μM ($n = 5$) for locus coeruleus neurones and $1{\cdot}9 \pm 0{\cdot}2$ μM ($n = 4$) for submucous neurones. Thus, despite the striking differences in the maximum shifts by cocaine of the noradrenaline responses observed between locus coeruleus and submucous plexus neurones, cocaine EC_{50} values were quite similar.

Effects of DMI on neuronal sensitivity to noradrenaline

Superfusion of locus coeruleus neurones with DMI (1 μM) produced essentially similar results to those obtained with cocaine; i.e. DMI increased the outward current produced by noradrenaline (see Williams *et al.* 1985). The onset of action of DMI was quite slow, requiring 20–30 min to reach steady-state levels. The noradrenaline

concentration–response curve was shifted to the left by 1 μM-DMI; the noradrenaline sensitivity ratios produced by 1 μM-DMI were 40·6 ± 9 ($n = 3$). This was not significantly different from that produced by 30 μM-cocaine. A higher concentration of DMI (10 μM) produced no further increase in the noradrenaline sensitivity of locus coeruleus neurones. The effects of DMI were not reversed within 5–7 h after washing DMI from the superfusion fluid and, after exposure to DMI, cocaine (1–100 μM) had no effect on the noradrenaline-induced outward current in these neurones. In submucous plexus neurones, the hyperpolarization or outward current produced by noradrenaline was either unchanged or decreased in amplitude by DMI (0·2–20 μM) ($n = 14$). However, DMI (2–20 μM) also reversibly depressed the hyperpolarization evoked by superfusion with 60 nM-somatostatin ($n = 3$) and 200 nM-met-enkephalin ($n = 2$), substances which have been shown to activate the same potassium conductance in submucous neurones as does noradrenaline (Mihara & North, 1986). Thus, DMI appears to directly alter post-synaptic membrane properties of submucous plexus neurones in addition to inhibiting neuronal catecholamine uptake. After washing DMI (2 μM) from the preparation for 20–40 min, the sensitivity to noradrenaline increased by 3 to 4·5-fold over the control dose–response curves obtained in the same neurones ($n = 3$). This increased sensitivity to noradrenaline after removal of DMI was not reversible within 5 h and, as was observed in locus coeruleus neurones, cocaine did not alter the responses to noradrenaline in neurones which had been exposed to DMI ($n = 6$). Therefore, irrespective of the obvious non-specific actions DMI has on the submucous neurones, these results do indicate that DMI also acts in an irreversible manner on the same uptake sites acted upon by cocaine.

DISCUSSION

We have previously shown that the i.p.s.p. and the hyperpolarization produced by exogenously applied noradrenaline in both locus coeruleus neurones and submucous plexus neurones are due to identical mechanisms; i.e. increased potassium conductance following post-synaptic α_2-adrenoceptor activation (Egan *et al.* 1983; Williams *et al.* 1985; North & Surprenant, 1985). The present study now provides information on the role neuronal noradrenaline uptake plays at these synapses.

Cocaine prolonged the i.p.s.p. and i.p.s.c. in neurones of the locus coeruleus and submucous plexus; it produced a maintained hyperpolarization or outward current in locus coeruleus cells and increased the amplitude and duration of spontaneous i.p.s.p.s in submucous plexus neurones. The effects of cocaine on the responses produced by superfusion with noradrenaline provided evidence that the prolongation of the inhibitory synaptic response was, in fact, due to blockade of noradrenaline uptake. That is, noradrenaline concentration–response curves constructed on these neurones in the absence and presence of cocaine allowed us to determine the concentration of cocaine which occupied all neuronal noradrenaline uptake sites; we found that 10–30 μM-cocaine saturated these uptake sites (Figs. 7 and 9). Prolongation of the synaptic response at cocaine concentrations greater than saturation values would indicate the actions of cocaine on the i.p.s.p. or i.p.s.c. were due to mechanisms other than noradrenaline uptake inhibition. In the present experiments, the prolongation of the inhibitory synaptic response and the shift in the noradrenaline

sensitivity ratio by cocaine occurred over the same concentration range with cocaine EC_{50} values being approximately the same for both effects. These results strengthen the conclusion that blockade of noradrenaline uptake sites by cocaine resulted in the prolongation of the i.p.s.p. or i.p.s.c.

The finding that cocaine produced greater and more variable shifts in the noradrenaline dose–response curves in locus coeruleus neurones than in submucous plexus neurones is most likely primarily due to differences in diffusion barriers which are present in these tissues. Diffusion barriers are minimal in the submucous plexus of the guinea-pig, where small numbers of neurones (one to thirty) lie flat against each other in a monolayer. No cells other than neurones are present within each of the ganglia, or nodes; the only diffusion barrier between the surface of the submucous neurone and the external solution appears to be the thin overlying membrane of the basal lamina (Wilson, Furness & Costa, 1981). In contrast, diffusion barriers may be considerable in the 300 μm slice of rat pons, where locus coeruleus and other neurones as well as glial cells are tightly packed in layers ten to twenty cells deep. Thus, the depth of the impaled neurone within the slice is probably the major factor contributing to the wide variability in the responses recorded from locus coeruleus neurones (see Nicholson & Hounsguard, 1983). This explanation is supported by the converse findings in submucous plexus neurones which showed remarkably little variation in maximum responses among cells. The same line of reasoning applies to the larger noradrenaline sensitivity ratios produced by cocaine in locus coeruleus neurones, in that a relatively larger proportion of added noradrenaline will be taken up in the brain-slice preparation in the absence of cocaine than in the submucous plexus.

When all neuronal uptake sites were blocked, the sensitivity to applied noradrenaline remained 10-fold greater in submucous plexus neurones than in locus coeruleus neurones (Fig. 8). This difference is somewhat artificial in that the dependent variable was the outward current induced by adding noradrenaline; however, cocaine itself caused an outward current in locus coeruleus neurones. This implies that some endogenous noradrenaline was continuously present at the post-synaptic α_2-adrenoceptors; this unknown amount likely contributed significantly to the total concentration when submaximal concentrations of exogenous noradrenaline were applied. On the other hand, it is also possible that differences in receptor reserve (i.e. 'spare' receptors) or in the contribution of non-neuronal uptake processes may account for these findings.

The mechanisms which determine the time course of these long-lasting (1–2 s) i.p.s.c.s are unknown. In the absence of cocaine, the duration of the i.p.s.c. in locus coeruleus and submucous plexus neurones is similar, suggesting that the rate of removal of noradrenaline from the extracellular space is similar at both of these synapses. In the locus coeruleus, where diffusion of noradrenaline is limited, cocaine markedly prolonged the i.p.s.c.; clearly reuptake of noradrenaline plays a major role in terminating (and determining) transmitter action at this synapse. In the submucous plexus, where diffusion is unhindered, the concentration of free noradrenaline present in the extracellular space may be assumed to be negligible within a few hundred milliseconds after its release (see Hartzell *et al.* 1977); here diffusion may be primarily responsible for terminating the actions of noradrenaline with reuptake playing a secondary role. That cocaine was effective in prolonging this i.p.s.c. implies that

noradrenaline can remain available throughout most of the time course of the synaptic response; this noradrenaline is probably bound to α_2-adrenoceptors or to non-specific binding sites. Thus, if the dissociation rate of noradrenaline from these sites is slow, cocaine will increase the probability of rebinding to the same (or nearby) receptor. The apparent fusing of spontaneous i.p.s.p.s in the presence of cocaine (Fig. 5) may be suggestive of a rebinding process.

Cocaine also increased the amplitude of the i.p.s.c. by approximately the same degree in both locus coeruleus and submucous plexus neurones. We cannot rule out the possibility that the increased amplitude was due to an increased mean channel open time of the activated potassium channels; however, it seems more reasonable to conclude that the increase in amplitude of the synaptic current resulted from a higher concentration of noradrenaline reaching the post-synaptic neurone following stimulation. These results indicate that a proportion of synaptically released noradrenaline may be taken up before it reaches the post-synaptic receptors. Alternatively, or additionally, cocaine may allow noradrenaline released from more distant varicosities to diffuse into 'effective' proximity with the post-synaptic membrane and its adrenoceptors.

Concentrations of cocaine (1–10 μM) which prolonged the inhibitory synaptic responses, increased neuronal sensitivity to exogenously applied noradrenaline and evoked a maintained outward current are close to plasma concentrations found after 'recreational' intravenous, intranasal or inhalational administration *in vivo* (Reith *et al.* 1983; Jones, 1984). Thus, it seems likely that enhancement of central adrenergic inhibitory synaptic potentials may contribute to the behavioural alterations occasioned by administration of this widely abused substance.

In conclusion, we have shown that synaptic potentials mediated by the release of noradrenaline at a central and at a peripheral adrenergic synapse are prolonged by inhibition of neuronal catecholamine uptake. Blockade of this uptake in those neuronal sites where noradrenergic tone is high can increase the effectiveness of spontaneously released noradrenaline to the extent of producing a sustained inhibition of neuronal activity.

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