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Actions of cocaine on rat nucleus accumbens neurones *in vitro*

N. Uchimura & R.A. North

Vollum Institute, Oregon Health Sciences University, Portland, Oregon 97201, U.S.A.

1 Intracellular recordings were made from 103 neurones of the rat nucleus accumbens *in vitro*.

2 Dopamine (3–100 μM; in sulpiride, 1 μM) hyperpolarized neurones (79%) by acting at D_1 receptors: dopamine (3–100 μM; in SCH23390, 1 μM) depolarized neurones (55%) by acting at D_2 receptors. 5-Hydroxytryptamine (1–100 μM) depolarized 86% neurones.

3 Both actions of dopamine as well as the effect of 5-hydroxytryptamine were potentiated by cocaine (0.3–30 μM), which had no effect of its own on membrane potential.

4 Dose-ratio was computed as [(concentration of agonist causing a 4 mV potential change in cocaine)/(concentration of agonist causing a 4 mV potential change without cocaine)]. Cocaine (1–30 μM) caused the same dose-ratio whether dopamine depolarizations (D_2) or hyperpolarizations (D_1) were measured; the dose-ratio ranged from 2 (1 μM) to 50 (30 μM).

5 Responses to 5-hydroxytryptamine were increased more than responses to dopamine; cocaine 1 μM gave a dose-ratio of 13.4 and at 30 μM gave a dose-ratio of 118.

6 It is concluded that cocaine acts to inhibit the uptake of dopamine and 5-hydroxytryptamine in slices of rat nucleus accumbens; lower concentrations of cocaine (0.3 to 1 μM) are particularly effective in potentiating the action of 5-hydroxytryptamine.

Introduction

The nucleus accumbens receives innervation from the dopamine-containing fibres originating in the ventral tegmental area and medial substantia nigra (Björklund & Hökfelt, 1984). Extracellular recording *in vivo* has shown that the main action of dopamine on rat nucleus accumbens neurones is inhibition (Woodruff *et al.*, 1976; White & Wang, 1986). This has been confirmed by *in vitro* experiments in the guinea-pig and rat in which controlled concentrations of agonists and antagonists were used (Higashi *et al.*, 1989; Uchimura *et al.*, 1986): the D_1 receptor is involved in this inhibition, activation of which increases the membrane potassium conductance. However, dopamine has an additional depolarizing action on a smaller group of nucleus accumbens neurones; this results from D_2 receptor activation and closure of potassium channels.

There is good evidence that an important site of action of cocaine is within the nucleus accumbens where it potentiates the action of released dopamine (see Koob & Bloom, 1988; Liebman & Cooper, 1989); the dopamine innervation of the accumbens must be intact for cocaine to be self-administered (Wise, 1984; Koob & Bloom, 1988). However, since cocaine is likely to act at many sites in the nervous system when administered systematically, it was thought of value to determine the concentrations of cocaine needed to potentiate actions of dopamine in isolated nucleus accumbens tissue. This was done in the present experiments by measuring the change in sensitivity to exogenous dopamine caused by various concentrations of cocaine, having first separated the two actions of dopamine (hyperpolarization and depolarization) with selective antagonists.

5-Hydroxytryptamine (5-HT)-containing fibres originating in the dorsal raphe also innervate the nucleus accumbens (Steinbusch, 1981), and 5-HT depolarizes and excites the neurones by reducing the conductance of inwardly rectifying potassium channels (North & Uchimura, 1989). Thus the predominant influences of dopamine and 5-HT on accumbens neurones are opposing; this is consistent with results of experiments which indicate that 5-HT decreases movements when administered into the nucleus (Pijenburg *et al.*, 1976) whereas dopamine agonists stimulate locomotion (Kelly & Roberts, 1983). Because cocaine also inhibits the uptake of 5-HT, it is possible that the potentiation of the actions of 5-HT in the accumbens also contributes to the overall effect of cocaine. A second purpose of the present experiments was to determine the concentrations of cocaine that were effective in potentiating the actions of 5-HT in the nucleus accumbens.

Methods

The methods used were similar to those previously described (Uchimura *et al.*, 1986; Higashi *et al.*, 1989; North & Uchimura, 1989). Rats (200–300 g) were anaesthetized with ether, and the brain was rapidly removed and then sliced frontally with a vibratome. A single slice (about 300 μm thick) containing a section of nucleus accumbens was transferred to a recording chamber (about 500 μl capacity) and submerged in a continuously flowing (3 ml min^{-1}) solution at 36°C. The solution contained (mM): NaCl 126, NaH_2PO_4 1.2, $MgCl_2$ 1.3, $CaCl_2$ 2.4, KCl 2.5, $NaHCO_3$ 26, glucose 10 and was gassed with 95% O_2 and 5% CO_2. Intracellular recordings were made from neurones lying within 500 μm medial to the anterior commissure, at a level of the nucleus accumbens that was about one-third of the distance along its rostral-caudal axis. The electrodes contained potassium chloride (2 M) and had resistances of 40–80 MΩ; both membrane potential and membrane current were measured (Axoclamp 2A amplifier). In voltage-clamp experiments the switching frequency was 2.5–3 kHz, and the head-stage potential was continuously monitored on a separate oscilloscope. The membrane current in response to slow ramp changes in membrane potentials (1 mV s^{-1}) was sometimes plotted directly onto an X–Y plotter; current and potential were plotted on a Gould 2400 pen recorder.

Drugs were applied to the tissue by changing this solution to one which differed only in its content of the drug. Because of the time required for the incoming solution to pass through a heat-exchanger, there was a delay of up to 1 min between the time of selecting the new solution and its first arrival at the tissue; the figures in this paper illustrate the times at which new solutions were selected. Drugs used were cocaine hydrochloride, dopamine hydrochloride, 5-hydroxytryptamine creatinine sulphate (Sigma), quinpirole (Lilly), SCH23390 (7-chloro-2,3,4,5-tetrahydro-3-methyl-5-phenyl-1H-3-benzazepine-7-ol maleate, Schering), SKF38393 (2,3,4,5-tetrahydro-7,8-dihydroxy-1-phenyl-1H-3-benzazepine hydrochloride, Smith Kline French) and (−)-sulpiride (Ravizza).

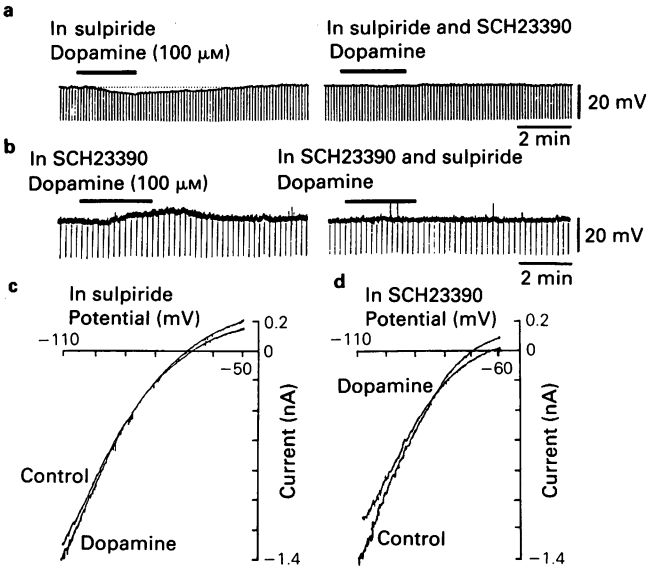

Figure 1 Receptor selective actions of dopamine. (a) In sulpiride (1 μM), dopamine hyperpolarizes a nucleus accumbens neurone (left trace). This is blocked by SCH23390 (1 μM, right trace). Control membrane potential, −75 mV. (b) In SCH23390 (1 μM), dopamine depolarizes a nucleus accumbens neurone (left trace). This is blocked by sulpiride (1 μM). Control membrane potential, −74 mV. In (a) and (b) the downward deflections are electrotonic potentials in response to a fixed current pulse (200 pA: 500 ms in a; 250 pA: 500 ms in b) repeatedly passed across the membrane. (c) Dopamine acts at a D_1 receptor to increase potassium conductance. Sulpiride (1 μM) present. Membrane currents (control and in 100 μM dopamine) were plotted in response to slow ramp depolarizing commands from −110 to −50 mV at 1–2 mV s^{-1}. (d) Dopamine acts at a D_2 receptor to close potassium channels. SCH23390 (1 μM) present. Membrane currents plotted as in (c). In (c) and (d) the extracellular potassium concentration was 6.5 mM.

Results

The present results are based on recordings made from 103 neurones, the properties of which were similar to those previously described for the rat nucleus accumbens (Higashi *et al.*, 1989; North & Uchimura, 1989). The resting potential of the neurones was −82.8 ± 0.9 mV (mean ± s.e.mean).

Actions of dopamine and 5-hydroxytryptamine

Dopamine has two effects on rat nucleus accumbens neurones (Uchimura *et al.*, 1986; Higashi *et al.*, 1989); about 50% of cells show a hyperpolarization followed by a depolarization, about 28% show only a hyperpolarization, and about 10% show only a depolarization. The predominance of the biphasic response to dopamine presented a problem in interpreting any potentiating action of cocaine, since the opposing effects might cancel each other. We therefore first confirmed that the two actions of dopamine could be separated with selective agonists and antagonists, prior to an examination of the effect of cocaine.

In the presence of sulpiride (1 μM), dopamine (3–100 μM) only hyperpolarized nucleus accumben neurones (n = 22 of 28 cells tested) (Figure 1a). In the presence of SCH23390 (1 μM), dopamine (3–100 μM) only depolarized the neurones (n = 25 of 45 cells) (Figure 1b). The antagonists themselves had no effect on the membrane potential. This selectivity was confirmed by experiments with other agonists; quinpirole (0.3–3 μM) caused a concentration-dependent depolarization (n = 7) whereas SKF38393 (0.3–3 μM) caused a concentration-dependent hyperpolarization (n = 8).

These actions of dopamine were studied under voltage clamp (n = 21). In the presence of sulpiride, dopamine caused an outward current at the resting potential which reversed to inward when the potential was hyperpolarized from the resting level (Figure 1c). In the presence of SCH23390, dopamine caused an inward current which reversed to outward when the membrane was hyperpolarized (Figure 1d). The observed reversal potentials conformed to the potassium equilibrium potential, as determined under similar conditions for the reversal of the dopamine-induced potential changes (Uchimura *et al.*, 1986; Higashi *et al.*, 1989) and for the reversal of the current induced by 5-HT (North & Uchimura, 1989).

The depolarization caused by 5-HT (n = 36 of 42 cells) was in all respects similar to that described previously (North & Uchimura, 1989).

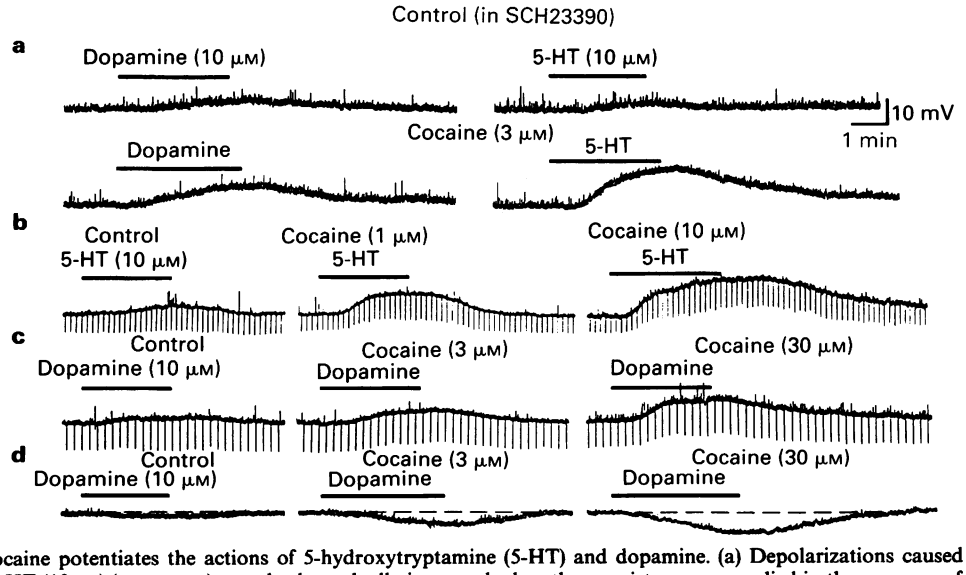

Figure 2 Cocaine potentiates the actions of 5-hydroxytryptamine (5-HT) and dopamine. (a) Depolarizations caused by dopamine (10 μM) and 5-HT (10 μM) (top traces) were both markedly increased when the agonists were reapplied in the presence of cocaine (3 μM) (bottom traces). The potentiation of the response to 5-HT was greater than for the response to dopamine. SCH23390 (1 μM) present throughout. Control membrane potential, −77 mV. (b) Potentiation of response to 5-HT (10 μM) by 1 μM and 10 μM cocaine. Control membrane potential, −76 mV. Downward deflections are electrotonic potentials, current pulse was 100 pA for 500 ms. (c) Potentiation of depolarizing (D_2) response to dopamine (10 μM) by 3 μM and 30 μM cocaine. SCH23390 (1 μM) present. Control membrane, −78 mV. Downward deflections are electrotonic potentials, current pulse was 150 pA for 500 ms. (d) Potentiation of hyperpolarizing (D_1) response to dopamine (10 μM) by 3 μM and 30 μM cocaine. Sulpiride (1 μM) present. Control membrane potential, −76 mV.

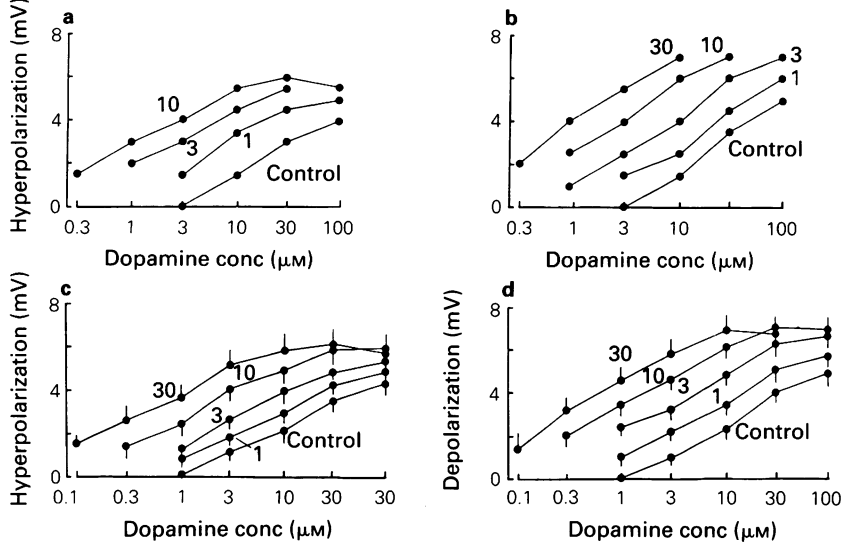

Figure 3 Potentiation of responses to dopamine. (a) and (b) Results from two neurones. (c) and (d) Results from many neurones (points are mean, with s.e.mean shown by vertical lines, 5–19 cells at each point). In each panel, the most righthand curves are the control responses to dopamine and the curves to the left of these were made in the presence of 1, 3, 10 and 30 μM cocaine as indicated. (a and c) Hyperpolarization (D_1) measured in sulpiride (1 μM). (b and d) Depolarization (D_2) measured in SCH23390 (1 μM). Membrane potential of neurones was held at −75 to −78 mV prior to dopamine application.

Cocaine potentiates effects of dopamine and 5-hydroxytryptamine

The actions of cocaine on the responses to dopamine and 5-HT are presented separately. However, some of the same cells were tested with both dopamine and 5-HT, before and after cocaine (e.g. Figure 2a). Cocaine (up to 10 μM) had no effect of its own on membrane potential; at 30 μM it caused a depolarization of up to 2 mV in 3 of 18 cells.

Hyperpolarization: D_1 receptors These experiments were done in the presence of sulpiride (1 μM). The hyperpolarization caused by dopamine was progressively potentiated as the concentration of cocaine was increased; this was evident during the course of recording from a single cell to which several different concentrations were applied (Figures 2 and 3), or by examining the averaged effects of dopamine/cocaine combinations on several cells (Figure 3). The minimum effective concentration of cocaine was 1 μM, and 10 μM caused a potentiation of approximately 21 fold (measured at 4 mV response level; see Figure 3).

Depolarization: D_2 receptors These experiments were done in the presence of SCH23390 (1 μM). The depolarization caused by dopamine was progressively potentiated by cocaine as the concentration was increased. This was clear from studying the action on individual cells (Figures 2 and 3), or from examining the average effect of dopamine in different cocaine concentrations (Figure 3). The threshold concentration of cocaine was 1 μM and a concentration of 10 μM increased the dopamine sensitivity by a factor of 19 (measured at the 4 mV level, Figure 3).

Depolarization: 5-HT_2 receptors Lower concentrations of 5-HT were needed to depolarize the accumbens neurones when cocaine was present. The potentiation of the action of 5-HT was obvious on individual cells (Figures 2 and 4), and was also clear from examination of the average effect of 5-HT in various concentrations of cocaine (Fig. 4). A concentration of 300 nM cocaine had a clear effect on the response to 5-HT, and with 10 μM cocaine the sensitivity was increased by a factor of 100 (measured at the 4 mV response level).

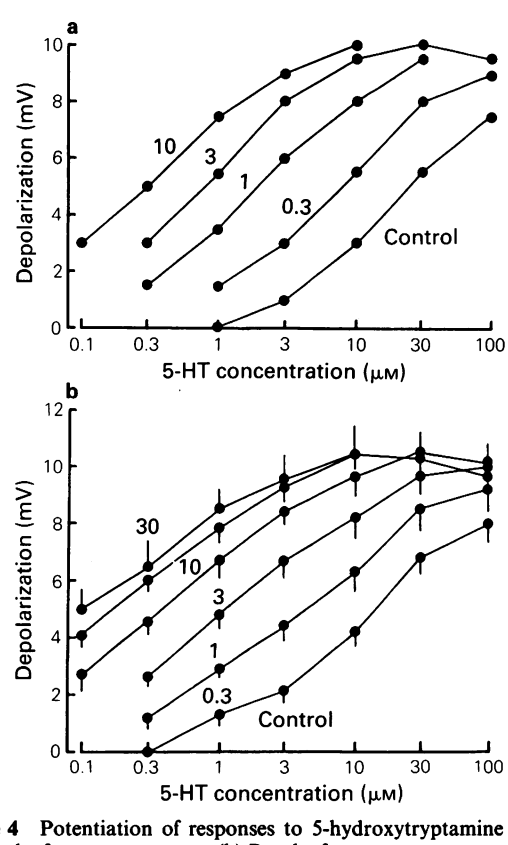

Figure 4 Potentiation of responses to 5-hydroxytryptamine (5-HT). (a) Results from one neurone. (b) Results from many neurones (points are mean with s.e.mean shown by vertical lines, 5–25 cells at each point). In each panel, the most righthand curve is the control response to dopamine and the curves to the left of these were made in the presence of (a) 1, 3, 10 and 30 μM cocaine and (b) 0.3, 1, 3, 10 and 30 μM cocaine as indicated. Membrane potential of neurones was held at −75 to −78 mV prior to 5-HT application.

Discussion

The main result is that cocaine increases the effectiveness of added dopamine or 5-HT in exerting their effects on individual nucleus accumbens neurones situated within a slice of

brain tissue. This is seen whether the effect is hyperpolarization (dopamine acting at D_1 receptors) or depolarization (dopamine acting at D_2 receptors or 5-HT acting at 5-HT_2 receptors; see North & Uchimura, 1989). It is assumed that the action of cocaine results from inhibition of uptake of the added agonists, and the subsequent discussion is within this framework.

Measurement of the ability of cocaine to block uptake

There is a method to estimate the dissociation constant for cocaine as an inhibitor of agonist uptake (K_i; Kenakin, 1987). However, it invokes a number of assumptions that are invalid in the present circumstances. First, it must be assumed that uptake sites and receptor sites are homogeneously distributed throughout the tissue. This is obviously not the case: the receptors under study are localized on a single neurone somewhere within the tissue, but the added agonists are exposed to uptake sites throughout the tissue as they diffuse toward the impaled cell. Thus, unless uptake is blocked one would expect a steep decline of agonist concentration from the surface of the slice to the neurone under study. This seems likely to contribute to the considerable variability in the sensitizing action of cocaine from cell to cell (Figure 5) (see also Surprenant & Williams, 1987).

Further assumptions of the Kenakin method for determining K_i are that the uptake process is described by a Michaelis-Menten formulation and that K_m (the dissociation constant for agonist at its uptake site) considerably exceeds the agonist concentration present. The second condition is not likely to be met. The concentrations of dopamine applied (100 μM) most likely exceed the K_m considerably; estimates of dopamine K_m measured biochemically are typically 100–1000 nM (e.g. Snyder & Coyle, 1969; Snyder *et al.*, 1970). A final difficulty is that the method of Kenakin (1987) requires the demonstration of a maximal sensitization; in most of the present experiments a clear maximal effect was not determined.

Comparison between inhibition of uptake of 5-hydroxytryptamine and of dopamine

Cocaine was no different in its ability to potentiate dopamine at D_1 receptors and dopamine at D_2 receptors; this may be interpreted to mean that the exogenous dopamine added to the slice undergoes a similar fate with respect to diffusion and uptake whether its eventual target is a D_1 or a D_2 receptor. It also implies that cocaine is not exerting any direct effects at one or other of the receptors. However, cocaine was significantly more effective in potentiating the action of 5-HT (Figure 2a and Figure 5). Since it was not possible to estimate a K_i value for cocaine (see above), the sensitization of the dopamine response was directly compared with the sensitization of the 5-HT response for the same four cocaine concentrations (Figure 5). At every concentration tested, cocaine was significantly more effective in increasing the effect of 5-HT. At 1 μM, cocaine was 6.5 times more effective in potentiating 5-HT than dopamine actions; at 30 μM the ratio was 2.5. This reduced differential effect at higher concentrations would be expected for a saturable uptake mechanism. The reasons for the differential effect cannot be determined: obvious possibilities are a different density of uptake sites (5-HT versus dopamine) or different K_i values for cocaine. The latter interpretation is consistent with the finding that the K_i for cocaine is about 4 fold lower when it is measured as an inhibitor of the binding of tritiated ligands at the 5-HT uptake site, than when measured as an inhibitor of binding to the dopamine site (Ritz *et al.*, 1987).

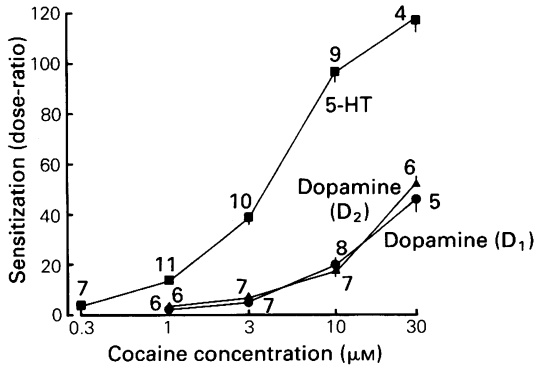

Figure 5 Degree of potentiation of responses to 5-hydroxytryptamine (5-HT) and dopamine caused by different concentrations of cocaine. Ordinate scale is dose-ratio [(concentration of agonist causing a 4 mV potential change in cocaine)/(concentration of agonist causing a 4 mV potential change without cocaine)]. Points show dose-ratio (mean with s.e.mean shown by vertical lines) for a number of individual neurones (indicated beside point): (■) 5-HT; (▲) dopamine at D_1 receptors (1 μM SCH23390 present); (●) dopamine at D_1 receptors (1 μM sulpiride present.)

Comparison with effects of cocaine in other tissues

Because cocaine had no effect when applied alone to the nucleus accumbens neurones, it is concluded that there is insignificant spontaneous release of dopamine (or 5-HT) occurring under these experimental conditions. This situation cannot be directly compared with the effects of cocaine in tissue slices containing cell bodies (noradrenaline in the locus coeruleus: Surprenant & Williams, 1987; 5-HT in the dorsal raphe: Williams & Lacey, 1988; dopamine in the substantia nigra/ventral tegmental area: Lacey *et al.*, 1990) because in those tissues cocaine itself has a strong hyperpolarizing effect through inhibiting the uptake of dopamine (or noradrenaline or 5-HT) that is being continuously released from the spontaneously firing neurones. The effect of cocaine in potentiating the actions of noradrenaline has been studied in a number of peripheral tissues that lack noradrenaline-containing cell bodies; in this case, the cocaine has little effect in the absence of applied noradrenaline. One such preparation is the submucous plexus isolated from the guinea-pig (Surprenant & Williams, 1987). In that tissue, the maximum sensitization by cocaine (measured as the leftward shift of the noradrenaline concentration-response curve) was only about 3.6 fold, compared with 120 fold (for 5-HT) or 50 fold (for dopamine) in the present experiments. The large difference between the tissues seems most likely to arise from the fact that few diffusional barriers exist in the isolated ganglia of the submucous plexus (which contain only 5–12 neurones) whereas agonist added to a brain slice must diffuse through as much as 200 μm of tissue laced with uptake sites before reaching the impaled neurone. This is mentioned as a reminder that the experimental conditions, particularly the opportunity for uptake provided by a long and tortuous diffusional pathway, play a very important role in determining the quantitative effect of an uptake blocker, as stressed by Kenakin (1987).

Those conditions should be equivalent with respect to 5-HT and dopamine in the present experiments and thus do not affect our conclusion that 5-HT uptake is more sensitive to cocaine than is dopamine uptake. However, they also emphasize that the large sensitizations to agonist which were observed are in a sense artifactual because of the long diffusional path which we have provided by recording from a neurone deep within a brain slice. 5-HT and dopamine released into the extracellular space during normal physiological activity would have their effects augmented much less by uptake inhibition. Direct measurements of the concentrations of dopamine (and cocaine) in dialysates of rat striatum extracellular fluid indicate that a change in the cocaine concentra-

tion of 1 μM is associated with a change in dopamine concentration of about 37 nM (Nicolaysen *et al.*, 1988), which is considerably less than the sensitization observed in the present study. The most appropriate way to approach the physiological circumstance may be to examine the effects of cocaine on the synaptic potentials mediated by the amine transmitters (Surprenant & Williams, 1987).

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