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Cocaine-sensitive uptake of sympathomimetic amines in nerve tissue

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The uptake of [^{3}H]octopamine, [^{3}H]norephedrine and [^{3}H]phenylethanolamine in slices of cerebral cortex and heart of the mouse was investigated. Cocaine inhibited the uptake of octopamine but had no effect on that of the other two amines, whose uptake seems to be a pure physico-chemical partition between the tissue and the solution. Together with previous results, these indicate that the cocaine-sensitive uptake is linked with the hydroxyl groups in the benzene nucleus and particularly with that in the *meta* position. The results bear out the view that the potentiating effect of cocaine on the direct effect of sympathomimetic amines is due to inhibition of the uptake of the amines in sympathetic nerves, but they contradict the assumption that the antagonizing effect of cocaine on the indirect action of the amines is due to inhibition of the amine uptake.

IN previous experiments it was found that the uptake of tritiated sympathomimetic amines in brain slices which had been incubated with the amines for a short time was greatly reduced by cocaine and desipramine but not by reserpine (Ross & Renyi, 1966a, b, c). This indicates that under these conditions the uptake of the amines via the neuron membranes of adrenergic nerves was the most important factor governing the amine accumulation. The rate of uptake was found to be dependent on the chemical structure of the amines. For instance noradrenaline and dopamine were taken up with similar velocities while tyramine showed a slower uptake. (−)-Amphetamine was not actively taken up at all.

To obtain more information the investigation has been extended with three amines, namely [^{3}H]octopamine, [^{3}H]norephedrine and [^{3}H]phenylethanolamine.

Experimental

MATERIALS AND METHODS

[^{3}H]Octopamine {(±)-*p*-[7-^{3}H]hydroxyphenylethanolamine; specific activity 2·5 c/mmole} was obtained from New England Nuclear Corp., USA. [^{3}H]Phenylethanolamine {(±)-[α-^{3}H]phenyl-β-aminoethanol; specific activity 110 mc/mmole} and [^{3}H]norephedrine {(±)-[α-^{3}H]-hydroxy-β-aminopropylbenzene; specific activity 100 mc/mmole} were synthesized by reducing α-aminoacetophenone and α-aminopropiophenone with tritiated sodium borohydride (The Radiochemical Centre, Amersham, England; specific activity 750 mc/mmole). The purity of the tritiated amines was checked by paper chromatography (n-butanol–acetic acid–water, 4 : 1 : 5).

The uptake of the amines in brain slices was determined as described previously (Ross & Renyi, 1966a). Unless otherwise specified the incubation solution contained 100 mg of brain slices (cerebral cortex of

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mouse) 0·2 nmole of the tritiated amine and the inhibitor to be tested in 2 ml of Krebs-Hensleit buffer containing 0·1% glucose. The incubation was made in an atmosphere of 93·5% oxygen and 6·5% carbon dioxide, at 37°. The tritiated amine was added to the solution after 5 min pre-incubation of the slices with or without the inhibitor.

The amount of the tritiated amine in the slices was measured as the total radioactivity in an ethanol extract of the homogenized slices. It was expressed in nmole/g of tissue. No attempt was made to separate metabolites formed in the tissue from the amine.

Results

Although the 3 amines were all accumulated in the slices above the external concentration, only the uptake of [^{3}H]octopamine was reduced by cocaine (Figs 1–3, Table 1). Pre-treatment of the animals with pheniprazine had scarcely any effect on the amount of [^{3}H]octopamine accumulated (Fig. 1), whereas reserpine effected a marked reduction with prolonged incubation (Table 2). These results indicate that the amine taken up is rapidly bound in noradrenaline storage granules.

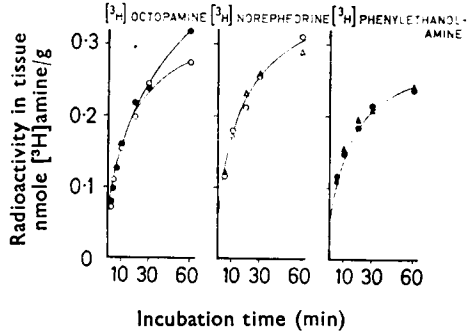


FIG. 1. Accumulation of [^{3}H]octopamine, [^{3}H]norephedrine and [^{3}H]phenylethanolamine (0·1 nmole/ml) in slices of mouse cerebral cortex (100 mg). —○— control slices. —△— slices with cocaine, 3×10^{-5}M. Filled symbols indicate pre-treatment with pheniprazine, 10 mg/kg i.p., 24 hr before the experiment.

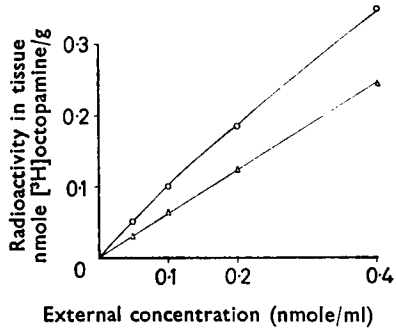


FIG. 2. Uptake of [^{3}H]octopamine in cortex slices at different external concentrations. Incubation time, 5 min. —○— control slices. —△— slices with cocaine, 3×10^{-5}M.

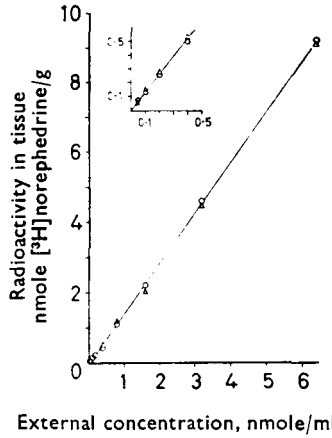


FIG. 3. Uptake of [^{3}H]norephedrine in cortex slices at different external concentrations. For conditions see legend to Fig. 2.

TABLE 1. INHIBITION OF THE UPTAKE OF [^{3}H]OCTOPAMINE IN SLICES OF CEREBRAL CORTEX OF THE MOUSE

	Conc. M	n	Uptake nmole/g in 5 min	-Δ Uptake nmole/g in 5 min
Control	—	6	0·119 ± 0·008	—
Cocaine	3×10^{-5}	3	0·075 ± 0·003*	0·044
	6×10^{-6}	3	0·082 ± 0·001*	0·037
	$1{\cdot}5 \times 10^{-6}$	3	0·089 ± 0·001*	0·030
	3×10^{-6}	3	0·100 ± 0·005	0·019
Desipramine.. ..	3×10^{-7}	3	0·078 ± 0·003*	0·041
	3×10^{-8}	3	0·089 ± 0·003*	0·030
	3×10^{-9}	3	0·094 ± 0·005	0·025

* $P < 0{\cdot}01$.

The slices were pre-incubated with the inhibitor for 5 min and with [^{3}H]octopamine (0·1 nmole/ml) for a further 5 min.

If the uptake of [^{3}H]octopamine in the first 5 min incubation is taken as the initial rate (Fig. 4), the approximate values of K_M and V_{max} for the cocaine-sensitive part of the uptake were 7×10^{-7}M and 0·06 nmole/g min. The value of K_M is about the same as that previously found for the uptake of noradrenaline, dopamine and tyramine. The value of V_{max} was close to that obtained for tyramine but lower than values for noradrenaline and dopamine (Ross & Renyi, 1966a).

TABLE 2. INHIBITION OF THE ACCUMULATION OF [^{3}H]OCTOPAMINE IN CEREBRAL CORTEX SLICES OF THE MOUSE BY RESERPINE

Incubation time (min)	Control	Reserpine (5 mg/kg)
5	0·111 ± 0·004	0·100 ± 0·004
30	0·310 ± 0·005	0·193 ± 0·014*

* $P < 0{\cdot}001$.

Reserpine was injected 18 hr before the experiment. The values are means ± s.e. for 4 animals.

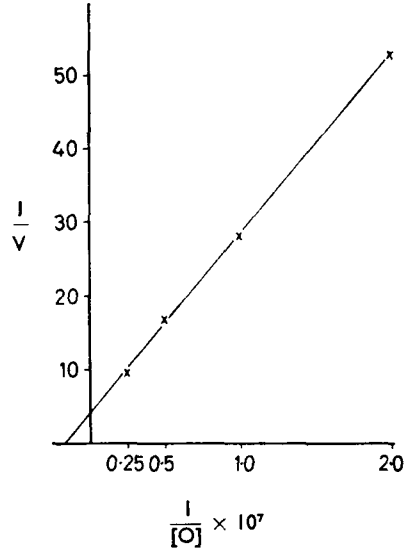


FIG. 4. Double reciprocal plot of the cocaine sensitive uptake of [^{3}H]octopamine in slices of mouse cerebral cortex. The plot is based on the experiments shown in Fig. 2. V = rate of uptake expressed in nmole/g and 5 min. The concentration of [^{3}H]octopamine [O] expressed in mole/litre.

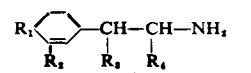
TABLE 3. ACCUMULATION OF [^{3}H]OCTOPAMINE, [^{3}H]NOREPHEDRINE AND [^{3}H]-PHENYLETHANOLAMINE IN HEART SLICES OF THE MOUSE

Amine	Accumulation of the amine nmole/g			
	n	Control	n	Cocaine 10 μg/ml
Octopamine	2	0·148 (0·152, 0·144)	2	0·074 (0·073, 0·076)
Norephedrine	4	0·244 ± 0·004	4	0·232 ± 0·004*
Phenylethanolamine ..	4	0·225 ± 0·012	4	0·205 ± 0·010*

* $P > 0·05$.

The heart slices were incubated with [^{3}H]amine (0·1 nmole/ml) for 15 min.

TABLE 4. RELATIONSHIP BETWEEN THE COCAINE SENSITIVE UPTAKE OF SYMPATHOMIMETIC AMINES AND THE EFFECT OF COCAINE ON THE DIRECT AND INDIRECT EFFECT OF THE AMINES



Amine	R_1	R_2	R_3	R_4	Uptake inhibited by cocaine	Direct action potentiated by cocaine*	Indirect action antagonized by cocaine*
Noradrenaline ..	OH	OH	OH	H	++	+	
Dopamine	OH	OH	H	H	++		
Metaraminol ..	H	OH	OH	Me	++	+	
Octopamine ..	OH	H	OH	H	+	−	+
Tyramine	OH	H	H	H	+		+
Norephedrine ..	H	H	OH	Me	−	−	+
Phenylethanolamine	H	H	OH	H	−	−	+
Amphetamine ..	H	H	H	Me	−		+

* According to Trendelenburg & others (1962).

Within a large concentration range the accumulation of [^{3}H]norephedrine in the slices was linear (Fig. 3); this indicates a pure physicochemical partition of the amine between the tissue and the solution.

The uptake of [^{3}H]octopamine, but not that of [^{3}H]norephedrine or [^{3}H]phenylethanolamines, was also inhibited in heart slices of the mouse by cocaine (Table 3).

Discussion

These experiments strengthen the earlier supposition of a relation between the chemical structure and the rate of uptake of sympathomimetic amines in brain tissue (Ross & Renyi, 1966a). As shown in Table 4 the cocaine-sensitive uptake seems to be linked with the hydroxyl groups in the benzene nucleus and in particular with that in the *meta* position. The hydroxyl group in the side-chain seems to be of secondary importance. Amines having no hydroxyl group in the benzene nucleus were not taken up by a cocaine-sensitive mechanism. Since similar results have been obtained for heart slices (Ross & Renyi, 1966b and this report) it seems probable that the relations obtained may be generally valid for the uptake of the amines in sympathetic nerves.

The similar pattern between the potentiating effect of cocaine on the direct component of the sympathomimetic effect of the amines described by Trendelenburg, Muskus & others (1962) and the cocaine-sensitive uptake of the amines bears out these authors' supposition that the potentiating action of cocaine is a result of inhibition of the uptake of the amine in sympathetic nerves. They found, however, that the direct action of octopamine (norsynephrine) on the nictitating membranes of the cat was not potentiated by cocaine. This observation may have its explanation in the rather weak direct action of this amine and its sensitivity to monoamine oxidase. Since the capacity of the uptake reaction is limited, it seems likely that the inactivation mechanism for those amines which had to be given in large doses is less dependent on the uptake reaction. It should be recalled that the action of the other more potent *p*-hydroxylated amines, or those that were metabolically more stable, studied by the above-mentioned authors, namely synephrine, *p*-hydroxyephedrine and *p*-hydroxyphenylpropanolamine, was significantly potentiated by cocaine.

Unlike the direct component of the sympathomimetic effect the indirect one is antagonized by cocaine (Trendelenburg, 1963). Among the amines listed in Table 4 the last 5 compounds exert an important indirect effect, and two of them—tyramine and amphetamine—act only indirectly (Trendelenburg, 1963). Our observations that three of these amines were not taken up by a cocaine-sensitive mechanism seems to contradict the supposition that the antagonizing effect of cocaine is due to inhibition of the uptake of these amines before they release noradrenaline (Trendelenburg, 1961). Ross & Renyi (1966a) proposed that the noradrenaline released by the indirectly-acting amines emanates from a small

extraneuronal store, possibly located in the carrier in the neuron membrane. However, the possibility also remains that the outward passage via the neuron membrane of noradrenaline released from an intraneuronal store is inhibited by cocaine.

Recent observations that in cat and rat the tachyphylaxis to the pressor effect of (+)-amphetamine, phenylethylamine, and mephentermine is only poorly or not at all crossed to tyramine and α-methyltyramine (Bhagat, 1965; Bhagat, Gordon & Kopin, 1965; Eble & Rudzik, 1965; Fawaz & Simaan, 1965; Day, 1967) indicate that the indirectly-acting amines may produce their effects by two distinct mechanisms (Day, 1967). It is interesting to note that the difference in chemical structure between these two groups of indirectly-acting amines is the same as that determining the uptake reaction discussed above.

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