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Uptake and metabolism of β-phenethylamine and tyramine in mouse brain and heart slices

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Tritium labelled β-phenethylamine and tyramine were incubated with slices of mouse brain and heart. Cocaine (3×10^{-5}M) caused a reduction of 30% in the uptake of tyramine as well as in the formation of its metabolite 4-hydroxyphenylacetic acid. Cocaine had no effect on either the uptake of phenethylamine nor its deamination to phenylacetic acid.

We have previously reported the uptake of various β-phenethylamine derivatives by slices of mouse brain and heart (Ross & Renyi, 1966; Ross, Renyi & Brunfelter, 1968). Cocaine reduces the uptake of those amines containing at least one phenolic hydroxyl group but has no effect on the uptake of amines without these hydroxyl groups. Since cocaine is known to be a potent inhibitor of the active uptake of catecholamines (Whitby, Herrting & Axelrod, 1960; Dengler, Spiegel & Titus, 1961; Hillarp & Malmfors, 1964) at the site of the neuronal membrane, it seems likely that the uptake of other phenethylamine derivatives which is sensitive to cocaine might utilize the same carrier mechanism as that used by catecholamines. The lack of inhibition by cocaine of the uptake of (+)-amphetamine, norephedrine and β-phenylethanolamine may indicate that these amines are not taken up by this mechanism (Ross & others, 1968). It has been suggested, however, that these amines are taken up by a cocaine-sensitive mechanism, but because of their non-polar nature they diffuse rapidly out of the cells while the more polar phenethylamines pass out only slowly (Thoenen, Hürlimann & Haefely, 1968). A rapid diffusion out could obscure an effect of cocaine.

To separate effects on uptake from effects on amine retention we have measured the enzymatic deamination of [4-^{3}H]β-phenylethylamine in brain and heart slices. Some of the monoamine oxidase (MAO) responsible for deamination is located in the adrenergic neurons (Roth & Stjärne, 1966; Champlain, Mueller & Axelrod, 1969). Thus, if an amine is actively taken up by cells by a cocaine-sensitive mechanism, the formation of phenylacetic acid should be decreased in the presence of cocaine. [^{3}H]Tyramine was used as a reference compound, since it is known to be actively taken up by the cocaine-sensitive mechanism (Ross & Renyi, 1966) and is a good substrate for MAO (Blaschko, 1952) yielding 4-hydroxyphenylacetic acid.

MATERIALS AND METHODS

[4-^{3}H]-β-Phenethylamine was synthetized from *p*-chloro-β-phenethylamine by catalytic exchange (Pd) of the chlorine atom with gaseous tritium (Isotoptjänst, AB Atomenergi, Studsvik, Sweden). The specific activity was 1·0 Ci/mmol. The radioactive purity was checked by paper chromatography in n-butanol–water–ethanol (40:10:10). [^{3}H]Tyramine (generally labelled; specific activity 7·3 Ci/mmol) was obtained from New England Nuclear Corp.

The incubation of brain (cerebral cortex) and heart slices from mouse with the tritium-labelled amines was as described by Ross & Renyi (1966). The incubation

mixture consisted of 0·2 nmol of the amine, 11 μmol of glucose, 100 mg of tissue slices and 0 or 60 nmol of cocaine. Incubations were in 2 ml of Krebs-Henseleit buffer, pH 7·4, in an atmosphere consisting of 6·5% carbon dioxide in oxygen. The slices were pre-incubated in the buffer for 5 min before the addition of the [^{3}H]amine. After 5 min (brain slices) or 10 min (heart slices) incubation the tissue were removed from the buffer, blotted on filter paper and homogenized in 1·0 ml of ethanol containing 100 μg of non-radioactive amine and 100 μg of the corresponding acid metabolite as carrier substances. A 1·0 ml aliquot of the incubation media was also saved. It was added to an equal volume of ethanol containing the appropriate carrier compounds.

Chromatographic separation of the amines was by means of thin-layer chromatography (PSC-Fertigplatten, Kieselgel F 254, Merck) in chloroform–formic acid–ethylacetate (35 : 10 : 55). Aliquots of 0·3 ml of the incubation media or 0·5 ml of the tissue extract were used. The compounds were localized by means of an ultraviolet light and were extracted from the silica gel in to 2 ml of Soluene-100 (Packard). Radioactivity was measured after the addition of 15 ml of scintillation liquid (PPO and POPOP in toluene).

RESULTS

Cocaine significantly reduced the rate of formation of 4-hydroxyphenylacetic acid from [^{3}H]tyramine on incubation with brain (Table 1) and heart slices (Table 2). The inhibition of 4-hydroxyphenylacetic acid formation was quantitatively similar to the inhibition of tyramine uptake. The total uptake of [^{3}H]tyramine is the sum of the amine found in the slices plus the 4-hydroxyphenylacetic acid formed. This

Table 1. *Effect of cocaine* (3×10^{-5}M) *on the formation of the* 4-*hydroxyphenylacetic acid* (4-HPAA) *from tyramine by slices from mouse brain.*

Treatment	n	Brain slices Tyramine nmol/g ± s.e.	Brain slices 4-HPAA nmol/g ± s.e.	Incubation fluid 4-HPAA nmol/g ± s.e.
None	23	0·055 ± 0·002	0·089 ± 0·002	0·345 ± 0·010
Cocaine ..	12	0·040 ± 0·003* (27%)	0·071 ± 0·003 (20%)	0·231 ± 0·009* (33%)

* $P < 0{\cdot}001$. 5 min incubation.

The figures are means ± standard error of mean. The percentage decrease produced by cocaine is indicated in brackets.

Table 2. *Effect of cocaine* (3×10^{-5}M) *on the formation of* 4-*hydroxyphenylacetic acid* (4-HPAA) *from tyramine by slices of mouse heart.*

Treatment	Heart slices Tyramine nmol/g ± s.e.	Heart slices 4-HPAA nmol/g ± s.e.	Incubation fluid 4-HPAA nmol/g ± s.e.
None	0·037 ± 0·002	0·119 ± 0·003	0·219 ± 0·007
Cocaine	0·026 ± 0·001* (30%)	0·084 ± 0·004* (29%)	0·145 ± 0·004* (34%)

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

10 min incubation. The figures are means ± standard error of mean from 12 determinations. The percentage decrease produced by cocaine is indicated in the brackets.

Table 3. *Effect of cocaine* (3×10^{-5}M) *on the formation of phenylacetic acid* (PAA) *from β-phenethylamine* (PEA) *by brain slices from mouse.*

Treatment	Brain slices PEA nmol/g ± s.e.	Brain slices PAA nmol/g ± s.e.	Incubation fluid PAA nmol/g ± s.e.
None	0·014 ± 0·001 (n = 18)	0·081 ± 0·003 (n = 34)	0·631 ± 0·033 (n = 16)
Cocaine	0·017 ± 0·002* (n = 18)	0·083 ± 0·004* (n = 18)	0·673 ± 0·023* (n = 16)

* $P > 0{\cdot}05$.

5 min incubation. The figures are means ± standard error of means from the number of determinations indicated in the brackets.

Table 4. *Effect of cocaine* (3×10^{-5}M) *on the formation of phenylacetic acid* (PAA) *from β-phenethylamine* (PEA) *by heart slices from mouse.*

Treatment	Heart slices PEA nmol/g ± s.e.	Heart slices PAA nmol/g ± s.e.	Incubation fluid PAA nmol/g ± s.e.
None	0·014 ± 0·001	0·123 ± 0·004	0·820 ± 0·022
Cocaine	0·012 ± 0·001*	0·121 ± 0·004*	0·888 ± 0·035*

* $P > 0{\cdot}05$.

10 min incubation. The figures are means ± standard error of mean from 12 determinations

uptake represents both active uptake and the result of passive diffusion. The assumption is made that the high level of cocaine used completely blocks active uptake. Thus, the net uptake of tyramine in the control brain slices was 0·489 nmol/g each 5 min. This value decreased to 0·342 nmol/g each 5 min in presence of cocaine, suggesting that the active uptake of tyramine amounts to 0·147 nmol/g each per 5 min at a concentration of [^{3}H]tyramine of 10^{-7}M. For heart slices under the same conditions this value is 0·120 nmol/g each 10 min.

The uptake and deamination of [^{3}H]β-phenylethylamine by brain and heart slices is shown in Tables 3 and 4. The total rate of uptake of phenethylamine in brain and heart slices was 0·726 nmol/g each 5 min and 0·957 nmol each 10 min, respectively. Cocaine had no significant effect on the uptake of this amine or on the formation of phenylacetic acid.

DISCUSSION

There are several problems that arise when one attempts to measure the uptake of a compound into a tissue, where it is being simultaneously metabolized (Ross & Renyi, 1969). In the present instance, since some of the metabolizing enzyme is located in the cells responsible for the uptake, inhibition of uptake should reduce the amount of the metabolite formed. The assumptions are made that cocaine specifically inhibits the active uptake of phenethylamines and that at a high concentration of cocaine active uptake approaches zero. The capacity of the active uptake mechanism can be estimated by summing the reduction in the formation of the metabolite with the decrease in the amount of the compound itself in the tissue.

The observation that the formation of 4-hydroxyphenylacetic acid was inhibited by cocaine to the same degree as the uptake of tyramine supports the assumption. Since neither the deamination nor the uptake of phenethylamine was inhibited by cocaine, this amine does not seem to be taken up by such a cocaine-sensitive mechanism. This observation supports the hypothesis that at least one phenolic hydroxyl group is necessary for the cocaine-sensitive uptake of phenethylamine derivatives (Ross & Renyi, 1966; Ross & others, 1968).

If the same relation between structure and active uptake described here for brain and heart tissue is also valid for uptake into the adrenergic nerves of other tissues it seems difficult to explain the antagonism by cocaine of the cardiovascular effects of indirectly acting amines, such as (+)-amphetamine and phenethylamine (Burn & Rand, 1958) as being due to an inhibition of the uptake of these indirectly-acting amines (Trendelenburg, 1961). It is possible that the cardiovascular effects are due to noradrenaline molecules released from sites located outside or at the neuron membranes for example the so called transfer site for which cocaine has high affinity (Ross & Renyi, 1966). Cocaine may also inhibit the outward passage of noradrenaline released from intraneuronal sites by the amines passively taken up. The possibility also exists that the cardiovascular effects of the indirectly acting amines are due to hydroxylated products of the amines.

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