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The effect of cocaine and imipramine on tyramine-induced release of noradrenaline-^{3}H from the rat vas deferens *in vitro*

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1. Tyramine 10^{-4}M significantly increased release of noradrenaline-7-^{3}H (NA-7-^{3}H) from rat vas deferens *in vitro*.

2. Neither cocaine 10^{-5}M nor imipramine 10^{-7}M–10^{-6}M significantly reduced tyramine-induced release of NA-7-^{3}H.

3. Increasing the exposure time to cocaine and imipramine from 10 to 20 min or pre-incubating the tissue with a wide range of NA-7-^{3}H concentrations (3·3–333·3 ng/ml.) did not affect the lack of inhibition by cocaine and imipramine.

4. It is suggested that the tyramine receptor in rat vas deferens differs from that in other systems and that blockade of tyramine-released noradrenaline at α-adrenergic receptors may be the most important mechanism for tyramine antagonism by imipramine-like drugs in this tissue.

Cocaine inhibits tyramine-induced contraction and tyramine-induced release of noradrenaline (NA) in cat aortae (Lockett & Eakins, 1960) and in isolated rabbit heart (Lindmar & Muscholl, 1961). There are reports indicating that cocaine does not inhibit tyramine-induced depletion of noradrenaline-7-^{3}H (NA-7-^{3}H) from rat heart *in vivo* (Hertting, Axelrod & Patrick, 1961 ; Bhagat & Gilliam, 1965), whereas Potter & Axelrod (1963), using a higher dose ratio of cocaine to tyramine than in the previous studies, found that cocaine did decrease the depletion of NA-7-^{3}H from rat heart by tyramine *in vivo*. Desipramine also has been reported to prevent tyramine-induced reduction of NA in rat heart (Kaumann & Basso, 1965). Previous work from this laboratory (Barnett, Glöge & Taber, 1967 ; Barnett, Symchowicz & Taber, 1968) has indicated that cocaine does not significantly affect the contractile response of the isolated rat vas deferens to cumulative concentrations of tyramine, whereas antidepressants such as imipramine completely abolished these responses. It has been postulated that imipramine-like drugs antagonize the contractile response of the isolated rat vas deferens by blocking the effect of released NA at α-adrenergic receptors rather than by preventing tyramine-induced release of NA (Barnett *et al.*, 1968). To evaluate this hypothesis further, we have studied the influence of cocaine and imipramine on tyramine-induced release of noradrenaline-7-^{3}H from the rat vas deferens *in vitro*.

Methods

Male albino rats (200–300 g in weight) were used in these experiments. Animals were killed by a blow to the head and the vas deferens was immediately dissected, cleaned and weighed. Tissues were then rinsed with cold Tyrode solution to remove residual blood and both vasa deferentia from each animal (average total weight, 110 mg) were placed in appropriate beakers containing 3 ml. of Tyrode solution. Noradrenaline-7-^{3}H (average specific activity 8·8 c/m-mole, New England Nuclear Corporation) in a volume of 0·01 ml. was added to all beakers with a micrometer syringe in amounts of 1·0, 0·05 or 0·01 μg. Incubation with shaking was carried out at 37° C for 15 min under an atmosphere of O_2 (95%) and CO_2 (5%).

After incubation, samples were removed from the incubator and placed in an ice bath. The tissues were separated rapidly from the media, blotted and washed in 3 ml. of cold Tyrode solution. After blotting, tissues were placed in 2·6 ml. of Tyrode solution to which 0·3 ml. of imipramine, cocaine, or Tyrode solution (Tyrode and tyramine control samples) were added. All samples were incubated again for 10 or 20 min. Tyramine (0·1 ml.) was then added to all beakers except the control sample (Tyrode control) and the incubation continued for an additional 5 min. Tissues were then washed in 3 ml. of Tyrode solution, blotted and placed in scintillation vials. The digestion was accomplished by allowing tissues to stand overnight in 2 ml. of NCS (Nuclear-Chicago solubilizing agent) at room temperature followed by a 6 hr incubation at 55° C. The samples were counted in a Packard Liquid Scintillation Spectrometer after the addition of 10 ml. of scintillation media consisting of 4 g PPO (2,5-diphenyloxazole) and 0·05 g of POPOP (1,4-bis-2-[4-methyl-5-phenyloxazolyl]-benzene) per l. of toluene. Aliquots of incubation media were treated in a similar fashion to that described for the tissue.

The tissue radioactivity content was expressed as NA-7-^{3}H, because previous experiments in the above conditions have shown that most of the radioactivity in the vas deferens consists of unchanged NA-7-^{3}H (Barnett *et al.*, 1968).

The concentration of tyramine used in this study (10^{-4}M) was chosen on the basis of previous studies (Barnett *et al.*, 1968) which showed that it produced a maximum or near-maximum (90–100%) contractile response of the isolated rat vas deferens. The concentrations of cocaine and imipramine used were selected on the basis of the aforementioned studies. The 5-min exposure time to tyramine corresponds to the maximum time needed to obtain a cumulative dose-response curve for the contractile response to tyramine, which was also determined in previous studies (Barnett *et al.*, 1968).

Solutions and drugs

The Tyrode solution had the following composition: NaCl 136·8 mM, KCl 2·7 mM, $MgCl_2$ 2·1 mM, $CaCl_2$ 1·8 mM, NaH_2PO_4 0·4 mM, $NaHCO_3$ 11·9 mM and glucose 5·5 mM. Tyramine, cocaine and imipramine were used as their hydrochloride salts. Drug concentrations are expressed in terms of their respective free bases.

Results

The results in Table 1 demonstrate the degree of net uptake obtained with three different concentrations of NA-7-^{3}H used to load the vas deferens. Appreciable

uptake was obtained with all three concentrations used and the degree of uptake found with the highest concentration (333·3 ng/ml.) closely agrees with that reported by Häggendal & Hamberger (1967). When uptake is expressed as a percentage of the radioactivity present in the incubation medium, the uptake with 333·3 ng/ml. of NA-7-^{3}H is less than with the lower concentrations (Table 1), indicating that the highest concentration used approaches saturation of the uptake mechanism.

TABLE 1. *Uptake of noradrenaline -7^{3}H in rat vas deferens* in vitro (*mean*±S.E.)

Total NA-7-^{3}H* in medium (ng)	Net uptake of NA-7-^{3}H (ng/100 mg tissue)	Net tissue uptake as % of NA-7-^{3}H in medium	Number of rats
10	1·8 ± 0·1	18·2 ± 0·7	56
50	7·0 ± 0·1	14·1 ± 0·3	20
1000	80·0 ± 1·9	8·0 ± 0·2	20

* The volume of incubation medium was 3ml. in each case.

TABLE 2. *Effect of cocaine and imipramine on tyramine-induced release of NA-7-^{3}H from isolated rat vas deferens*

Treatment	NA-7-^{3}H release* (mean±S.E.)		
	Experiment A †	Experiment B †	Experiment C †
Tyrode control	34·0 ± 1·1	18·2 ± 0·8	20·3 ± 1·1
Tyramine 10^{-4}M control	38·8 ± 0·9 ‡	27·0 ± 0·9 ‡	31·1 ± 0·9 ‡
Cocaine 10^{-5}M + tyramine 10^{-4}M	40·4 ± 0·8 §	27·8 ± 1·5 §	34·7 ± 0·5 §
Imipramine 10^{-7}M + tyramine 10^{-4}M	37·3 ± 1·5 §	28·7 ± 1·1 §	32·1 ± 0·2 §
Imipramine 10^{-6}M + tyramine 10^{-4}M	—	—	32·1 ± 0·9 §

Four rats were used for each treatment in each experiment; tissues were exposed to test drugs for 10 min before adding tyramine.

* Release of NA-7-^{3}H is expressed as % of total NA-7-^{3}H originally in tissue=

$$100 \times \frac{\text{c.p.m. NA-7-}^3\text{H in medium}}{\text{total c.p.m. NA-7-}^3\text{H (medium + tissue)}}$$

† Experiment A: Vas deferens was pre-incubated with NA-7-^{3}H 333·0 ng/ml.
Experiment B: Vas deferens was pre-incubated with NA-7-^{3}H 16·5 ng/ml.
Experiment C: Vas deferens was pre-incubated with NA-7-^{3}H 3·3 ng/ml.

‡ Significantly different from Tyrode control, $P < 0·05$ in experiment A and $P < 0·01$ for experiments B and C, using analysis of variance.

§ Significantly different from Tyrode control, $P < 0·05$, but not from tyramine control, $P > 0·05$, using analysis of variance.

TABLE 3. *Effect of cocaine and imipramine* on tyramine-induced release of NA-7-^{3}H from isolated rat vas deferens*

Treatment	NA-7-^{3}H release * (mean±S.E.)
Tyrode control	29·0 ± 0·8
Tyramine 10^{-4}M control	39·4 ± 0·7 †
Cocaine 10^{-5}M + tyramine 10^{-4}M	37·6 ± 1·2 ‡
Imipramine 10^{-6}M + tyramine 10^{-4}M	37·7 ± 0·9 ‡

Tissue was exposed to test drug for 20 min before adding tyramine. Ten rats were used for each treatment.

* Vas deferens was pre-incubated with NA-7-^{3}H 3·3 ng/ml.; NA-7-^{3}H release is expressed as % of total NA-7-^{3}H originally in tissue (see Table 1).

† Significantly different from Tyrode control, $P < 0·05$, analysis of variance.

‡ Significantly different from Tyrode control, $P < 0·05$, but not from tyramine control, $P > 0·05$, analysis of variance.

Tyramine significantly increased the percentage of radioactivity released into the incubation medium (Table 2). The NA released by tyramine alone (tyramine control minus Tyrode control) was greatest when the tissue was pre-incubated with the lowest concentration of NA-7-^{3}H (experiment C). The degree of spontaneous release of NA-7-^{3}H (Tyrode control) after incubation with the highest concentration of NA-7-^{3}H (experiment A) was approximately twice that obtained with the other two pre-incubation concentrations. This high spontaneous release (experiment A) probably accounts for the relatively low degree of net release observed with tyramine. Regardless of the percentage of radioactivity released by tyramine, however, cocaine 10^{-5}M did not significantly reduce tyramine-induced release of NA-7-^{3}H (Table 2). Likewise, imipramine 10^{-7}M and 10^{-6}M—concentrations which effectively inhibited the tyramine-induced contractile response of the isolated vas deferens (Barnett *et al.*, 1968)—did not significantly reduce tyramine-induced release of NA-7-^{3}H from this tissue.

Since the contractile studies (Barnett *et al.*, 1968) involved incubation with cocaine and imipramine for 20 min and that of the present biochemical studies for only 10 min (to minimize spontaneous release of NA-7-^{3}H) additional experiments were performed to ascertain the effect of duration of incubation. Increasing the incubation period to 20 min did not affect the results; neither cocaine nor imipramine significantly decreased tyramine-induced release of NA-7-^{3}H (Table 3).

Discussion

There is evidence to substantiate the conclusion that both the uptake of NA and tyramine-induced release of NA are specific for adrenergic neurones of the rat vas deferens. We have previously demonstrated (Barnett *et al.*, 1968) that the uptake *in vitro* of NA-7-^{3}H (333·3 ng/ml.) can be significantly depressed by the concentrations of cocaine and imipramine used in the present study. Häggendal & Hamberger (1967) also showed that desipramine inhibited the uptake of a comparable concentration of NA in rat vas deferens. In the present investigation, the specificity of tyramine-induced NA release is suggested by the relatively constant NA release by tyramine 10^{-4}M despite varying the pre-incubation concentration of NA-7-^{3}H by a factor of 5 (3·3–16·5 ng/ml., Table 2).

The fact that cocaine does not inhibit either tyramine-induced contractions or tyramine-induced release of NA-7-^{3}H from rat vas deferens indicates that the tyramine receptor of this tissue differs from that in other systems, such as rabbit heart (Lindmar & Muscholl, 1961) and cat aorta (Lockett & Eakins, 1960). Yet in the vas deferens, as in these other tissues, the contractile response to tyramine is related to catecholamine release. Thus reserpine pretreatment causes the complete disappearance of NA from adrenergic nerve terminals of rat vas deferens (Taxi & Droz, 1966) and completely abolishes the contractile response to tyramine (Patil, LaPidus, Campbell & Tye, 1967).

A major question to be answered, therefore, is how imipramine-like drugs, but not cocaine, can inhibit tyramine-induced contractions of isolated rat vas deferens without decreasing tyramine-induced release of NA. Imipramine-like drugs in contrast to cocaine have α-adrenergic blocking activity (Brodie, Dick, Kielholz, Poldinger & Theobald, 1961; Ursillo & Jacobson, 1965; Barnett *et al.*, 1968) which probably accounts for their ability to inhibit the contractile response to tyramine in isolated

rat vas deferens. As indicated previously (Barnett *et al.*, 1968), phentolamine is virtually equipotent to imipramine and desipramine in abolishing the contractile response of this tissue to tyramine. Moreover, phentolamine produces non-competitive inhibition of tyramine in vas deferens as do imipramine-like drugs, whereas cocaine inhibits tyramine competitively in other preparations such as rat blood pressure (Bonaccorsi & Garattini, 1966) and cat nictitating membrane (Trendelenburg, 1961). Thus it is apparent that the tyramine receptor in rat vas deferens differs from that in other tissues. Anatomically, the vas deferens has also been shown to differ from other adrenergically innervated tissues such as heart and spleen because it contains relatively short adrenergic neurones (Sjöstrand, 1965). Short adrenergic neurones are characteristic of both male and female reproductive organs (Sjöstrand, 1965 ; Owman, Rosengren & Sjöberg, 1966, 1967 ; Owman & Sjöstrand, 1965). This may account for the pharmacological differences found between vas deferens and other sympathetically innervated tissues in our work as well as that of other investigators (Euler & Lishajko, 1966 ; Iverson, Glowinski & Axelrod, 1966 ; Sjöstrand & Swedin, 1968 ; Stjärne & Lishajko, 1966).

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