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The effects of drugs inhibiting catecholamine uptake on tyramine and noradrenaline-induced contractions of the isolated rat vas deferens

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1. Cocaine did not antagonize the tyramine-induced contractile response of the isolated rat vas deferens at the same concentrations which markedly potentiated the contractile response to noradrenaline.

2. Imipramine and amitriptyline non-competitively antagonized the contractile response to tyramine but did not potentiate noradrenaline. Desmethylimipramine produced both potentiation of noradrenaline and antagonism of tyramine.

3. Dexchlorpheniramine non-competitively antagonized the contractile response to tyramine. It also produced an atypical potentiation of noradrenaline in which lower concentrations of noradrenaline were potentiated to a greater extent than higher ones.

4. Imipramine inhibited the *in vitro* uptake of noradrenaline-^{3}H in rat vas deferens as did cocaine, desmethylimipramine and dexchlorpheniramine. These results suggest that the α-adrenergic blocking property of imipramine masks the potentiation of noradrenaline by uptake inhibition.

5. Evidence is also presented which suggests that α-adrenergic blockade of released noradrenaline may be the major mechanism for tyramine inhibition by imipramine-like drugs. This may explain why cocaine, which has no real alpha blocking action, is ineffective against tyramine.

Cocaine produces a combination of noradrenaline (NA) potentiation and tyramine antagonism in systems such as rat blood pressure (Bonaccorsi & Garattini, 1966). Tricyclic antidepressant drugs such as imipramine and amitriptyline, and antihistamines such as dexchlorpheniramine have also been found to have cocaine-like effects (Osborne & Sigg, 1960 ; Isaac & Goth, 1964 ; Stone, 1966 ; Johnson & Kahn, 1966). Furchgott, Kirpekar, Rieker & Schwab (1963), and more recently Johnson & Kahn (1966), have attributed both these effects to inhibition of NA and tyramine uptake by sympathetic nerve endings. Thus it is thought that blockade of uptake enhances the response to NA by preventing its major means of inactivation. This mechanism may also block the response to tyramine by preventing it from reaching its receptor site. The effect of various drugs which interfere with catecholamine uptake has been studied on NA and tyramine-induced contractions of the isolated rat vas deferens. On the basis of these contractile studies, effective

concentrations of cocaine, imipramine, desmethylimipramine and dexchlorpheniramine were selected to study whether the changes in contractile response to noradrenaline produced by effective concentrations of these drugs would correlate with inhibition of noradrenaline-^{3}H uptake in similar conditions.

Methods

Male Sprague-Dawley rats, 200–300 g in weight, were killed by a blow on the head and the right vas deferens was immediately excised, placed in Tyrode solution and aerated with a mixture of 95% oxygen and 5% carbon dioxide at a constant temperature of 37° C. Isometric contractile force was measured and recorded using a Grass FT 10 transducer and an Offner Type R dynograph. After equilibration for 1 hr, a series of cumulative dose-response curves were obtained for NA and tyramine in the presence and absence of antagonist. Cumulative dose-response curves with NA and tyramine were obtained by starting with a concentration of 10^{-7}M (10^{-6}M for tyramine), increasing the concentration by 0.5 log unit increments (after the force of contraction reached a constant level for each concentration) until addition of NA or tyramine produced no further increase (less than 5%) in force of contraction. In each experiment eight cumulative dose-response curves, four each for noradrenaline and tyramine, were determined. Curves for noradrenaline were alternated with those for tyramine. The first four and last two curves were controls, while curves five and six were determined in the presence of an antagonist drug. Preliminary experiments indicated that alternating the administration of NA with tyramine with washouts after each curve did not affect subsequent NA or tyramine curves. In addition, experiments were performed using only NA or tyramine but not both, and the effects of test drugs did not differ from those in experiments in which both NA and tyramine were used. Ten minutes were allowed between curves. An incubation time of 20 min was used for test drugs before obtaining either the NA or tyramine curves in the presence of test drugs, because it has been reported that this time is optimal for potentiation of NA by desmethylimipramine (DMI) (Ursillo & Jacobson, 1965). Lengthening the interval between curves to 20 min without antagonist did not affect subsequent NA or tyramine responses. The dose-response curves were plotted as a percentage of individual maximum response for NA rather than as a percentage of control NA response, so that drug-induced increases in maximal cumulative response to NA are not shown (Barnett, Greenhouse & Taber, 1968).

For the *in vitro* uptake studies, the vas deferens was excised in the same manner and incubated in Tyrode solution containing antagonist for 20 min at 37° C in an atmosphere of 95% oxygen and 5% carbon dioxide. At that time, noradrenaline-7-^{3}H (specific activity 4.37 c/m-mole, New England Nuclear Corp.) was added to the incubation medium to make a final concentration of 2×10^{-6}M. After 15 min the tissues were removed from the incubation medium, washed with Tyrode solution and treated with 2 ml. of NCS solubilizer (Nuclear-Chicago). Ten millilitres of scintillation medium (consisting of 4 g of PPO and 50 mg of dimethyl POPOP/l. of toluene) was then added to the digested tissue and the total radioactivity counted in a Packard liquid scintillation spectrometer.

Pilot studies have shown that the rate of uptake of NA-^{3}H (2×10^{-6}M) at 15 min is similar to that at 5 min, the latter being the approximate time for obtaining a cumulative dose-response curve for the NA contractile response. The actual uptake

of NA-^{3}H was 8.3 ± 0.2% of the NA-^{3}H in the incubation medium, which corresponds to 830 ng/g of tissue. A similar degree of NA uptake into vas deferens was reported by Häggendal & Hamberger (1967). Preliminary experiments using an ethanol extraction procedure and paper chromatography (Symchowicz & Korduba, 1967) indicate that 73% of NA-^{3}H incorporated in tissue is in the form of unchanged NA-^{3}H. The remainder of the radioactivity (at 15 min) consists chiefly of acidic metabolites.

Results are expressed as c.p.m./mg tissue and the percentage of inhibition was calculated by the formula 100% minus the ratio of uptake of radioactivity in test samples to that of control samples. Analysis of variance was used to determine statistical significance.

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. The following drugs were used: (−)-noradrenaline bitartrate, desmethylimipramine, cocaine hydrochloride, dexchlorpheniramine maleate, imipramine hydrochloride, amitriptyline hydrochloride and phentolamine methanesulphonate.

Results

Cocaine did not significantly affect the contractile response to tyramine at concentrations (10^{-6} to 10^{-5}M) which markedly potentiated the response to NA (Fig. 1). Concentrations of cocaine greater than 10^{-5}M caused contractions of the vas deferens and thus could not be used.

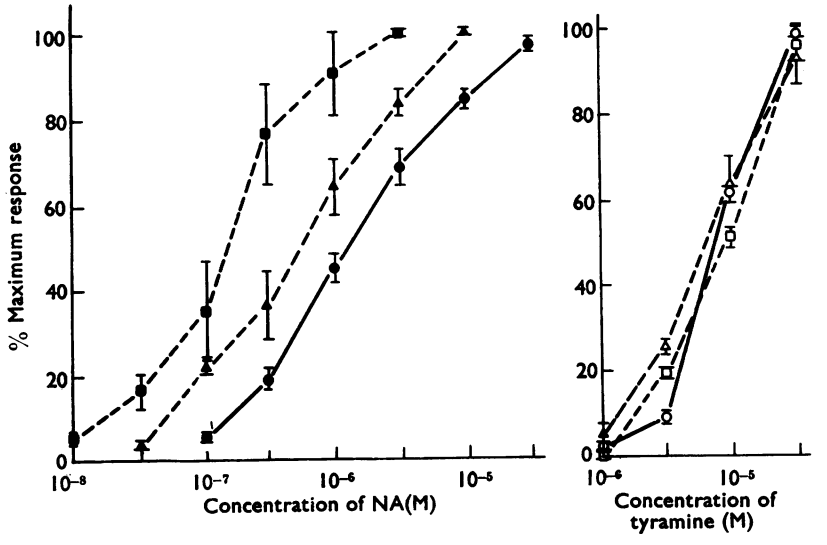

FIG. 1. Effect of cocaine on the contractile responses of the isolated rat vas deferens to NA and tyramine, expressed as per cent of individual maximum response for NA curves and per cent of control maximum response for tyramine curves. ●, Control NA; ▲, NA+cocaine 10^{-6}M; ■, NA+cocaine 10^{-5}M; ○, control tyramine; △, tyramine+cocaine 10^{-6}M; □, tyramine+cocaine 10^{-5}M. Each control curve represents the mean ± S.E. of six experiments. Each curve in the presence of drug represents the mean ± S.E. of three experiments.

The most selective effect of imipramine, however, was tyramine antagonism (Fig. 2). Imipramine 10^{-7}M reduced the maximum response to tyramine by 20–30% and left the NA response unaffected. Higher concentrations of imipramine completely abolished the tyramine response and shifted the NA curve to the right, the latter presumably indicating alpha adrenergic receptor blockade. Similar results were obtained with amitriptyline (Fig. 3). Noradrenaline potentiation was not seen with either imipramine or amitriptyline in concentrations from 10^{-10} to 10^{-6}M. In contrast, DMI did potentiate NA (Fig. 4), thus confirming the results of Ursillo & Jacobson (1965). DMI 10^{-8}M shifted the NA curve to the left by 0.5 log units; 10^{-7}M did not shift the NA curve any further to the left, whereas DMI 10^{-6}M actually produced less of a shift to the left than did DMI 10^{-7} or 10^{-6}M. The latter finding was also reported by Ursillo & Jacobson (1965) and indicates that the potentiation of NA by DMI is probably limited by concurrent alpha adrenergic blockade. DMI in the same concentrations (10^{-8} to 10^{-6}M) significantly reduced the maximum response to tyramine.

Dexchlorpheniramine also reduced the maximum contractile response to tyramine, but only at concentrations greater than 10^{-5}M. Dexchlorpheniramine 10^{-5} to 10^{-4}M produced an unusual pattern of NA potentiation in that the degree of enhancement was not uniform, and this resulted in a non-parallel shift of the NA curve to the left (Fig. 5). The same results (not shown) were found using the racemic mixture (chlorpheniramine).

The influence of an alpha-adrenergic blocking agent, phentolamine, on the tyramine and NA dose-response curves also was examined. Phentolamine reduced the maximum response to tyramine in the same concentrations as imipramine, DMI or amitriptyline (Fig. 6). As expected, phentolamine produced a competitive inhibitory shift of the NA curve to the right.

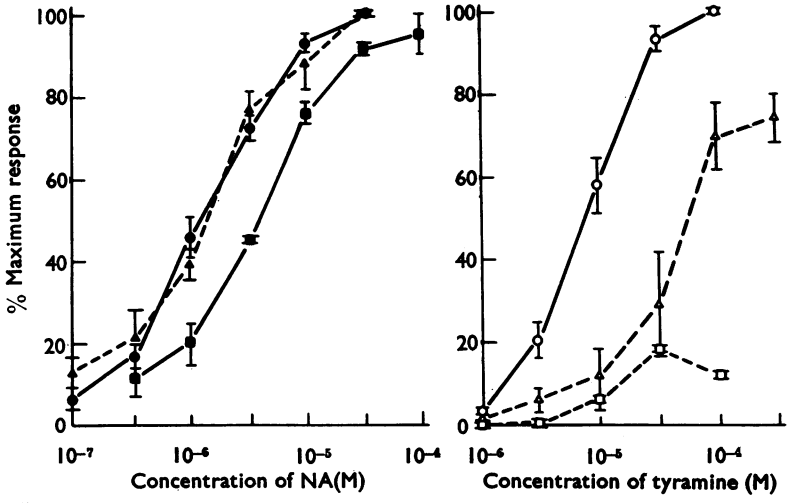

FIG. 2. Effect of imipramine on the contractile responses of the isolated rat vas deferens to NA and tyramine, expressed as per cent of individual maximum response for NA curves and per cent of control maximum response for tyramine curves. ●, Control NA; ▲, NA+imipramine 10^{-7}M; ■, NA+imipramine 10^{-6}M; ○, control tyramine; △, tyramine+imipramine 10^{-7}M; □, tyramine+imipramine 10^{-6}M. Each control curve represents the mean ±S.E. of six experiments. Each curve in the presence of drug represents the mean±S.E. of three experiments.

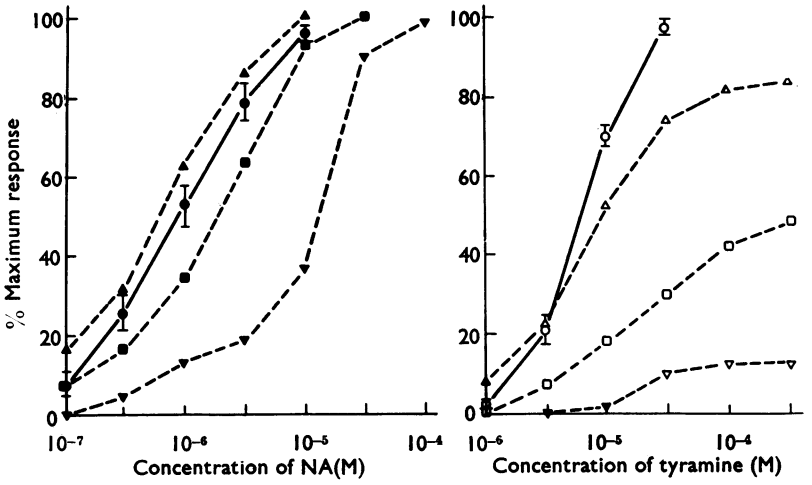

FIG. 3. Effect of amitriptyline on the contractile responses of the isolated rat vas deferens to norepinephrine and tyramine, expressed as per cent of individual maximum response for NA curves and per cent of control maximum response for tyramine curves. ●, Control NA; ▲, NA+amitriptyline 10^{-8}M; ■, NA+amitriptyline 10^{-7}M; ▼, NA+amitriptyline 10^{-6}M; ○, control tyramine; △, tyramine+amitriptyline 10^{-8}M; □, tyramine+amitriptyline 10^{-7}M; ▽, tyramine+amitriptyline 10^{-6}M. Each control curve represents the mean±S.E. of six experiments. Each curve in the presence of drug represents the mean of two experiments.

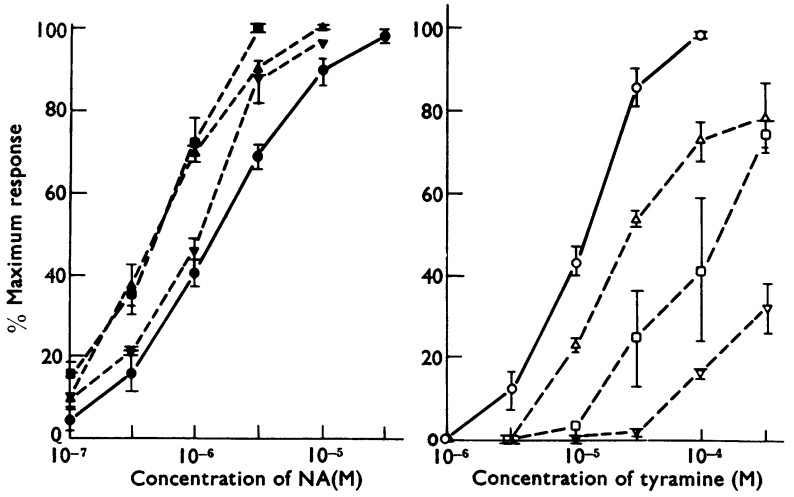

FIG. 4. Effect of desmethylimipramine (DMI) on the contractile response of the isolated rat vas deferens to NA and tyramine, expressed as per cent of individual maximum response for NA curves and per cent of control maximum response for tyramine curves. ●, Control NA; ▲, NA+DMI 10^{-8}M; ■, NA+DMI 10^{-7}M; ▼, NA+DMI 10^{-6}M; ○, control tyramine; △, tyramine+DMI 10^{-8}M; □, tyramine+DMI 10^{-7}M; ▽, tyramine+DMI 10^{-6}M. Each control curve represents the mean±S.E. of nine experiments. Each curve in the presence of drug represents the mean±S.E. of three experiments.

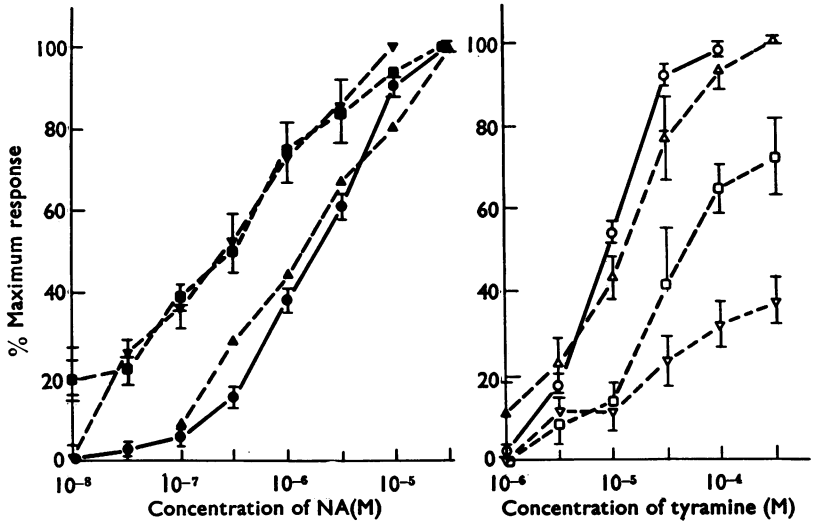

FIG. 5. Effect of dexchlorpheniramine on the contractile responses of the isolated rat vas deferens to NA and tyramine, expressed as per cent of individual maximum response for NA curves and per cent of control maximum response for tyramine curves. ●, Control NA; ▲, NA+dexchlorpheniramine 10^{-6}M; ■, NA+dexchlorpheniramine 10^{-5}M; ▼, NA+dexchlorpheniramine 10^{-4}M; ○, control tyramine; △, tyramine+dexchlorpheniramine 10^{-5}M; □, tyramine+dexchlorpheniramine 3×10^{-5}M; ▽, tyramine+dexchlorpheniramine 10^{-4}M. Each control curve represents the mean±S.E. of nine experiments. Each curve in the presence of drug represents the mean±S.E. of three experiments.

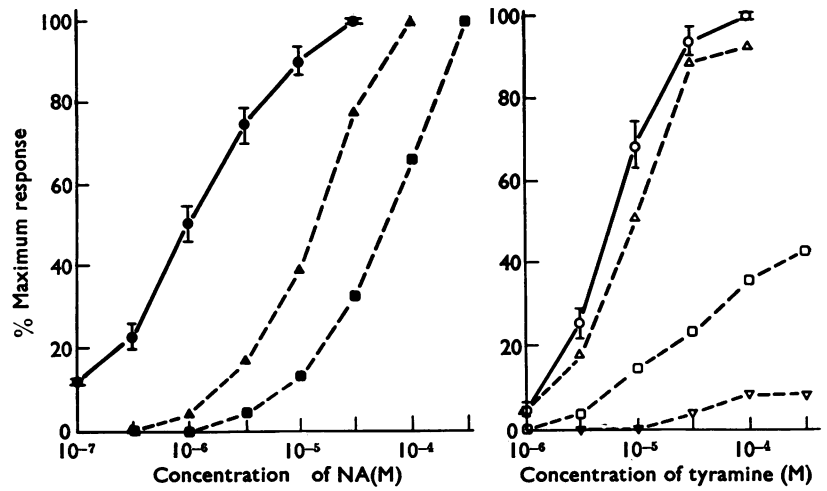

FIG. 6. Effect of phentolamine on the contractile responses of the isolated rat vas deferens to NA and tyramine, expressed as per cent of individual maximum response for tyramine curves. ●, Control NA; ▲, NA+phentolamine 10^{-6}M; ■, NA+phentolamine 10^{-5}M; ○, control tyramine; △, tyramine+phentolamine 10^{-8}M; □, tyramine+phentolamine 10^{-7}M; ▽, tyramine+phentolamine 10^{-6}M. Each control NA curve represents the mean±S.E. of four experiments, whereas each control tyramine curve represents the mean±S.E. of six experiments. Each curve in the presence of drug represents the mean of two experiments.

The effect of imipramine 10^{-7}M on uptake of NA-^{3}H was examined *in vitro* to investigate whether imipramine blocks NA uptake in rat vas deferens even though it does not produce potentiation of the NA contractile response. This concentration of imipramine (10^{-7}M) was chosen because it was effective in antagonizing the contractile response to tyramine and also because this concentration of DMI potentiated noradrenaline. The effects of cocaine and dexchlorpheniramine on NA-^{3}H uptake were also studied. Both drugs at a concentration of 10^{-5}M produced significant potentiation of NA. The concentration of NA-^{3}H (2×10^{-6}M) was chosen because it was the mean ED50 for all the *in vitro* contractile experiments as determined by interpolation of each of the NA control curves. The results in Table 1 indicate that imipramine significantly ($P<0.05$, analysis of variance) reduced uptake of NA-^{3}H as did DMI, cocaine and dexchlorpheniramine ($P<0.05$ for each drug). The inhibition of NA-^{3}H uptake by DMI is in agreement with the work of Häggendal & Hamberger (1967) in rat vas deferens.

Discussion

The lack of antagonism by cocaine of the contractile response to tyramine indicates that the tyramine receptor in the rat vas deferens is unique. Preteatment with reserpine abolishes the contractile response to tyramine (Patil, La Pidus, Campbell & Tye, 1967) as does phentolamine, so that it can be assumed that the major part of the contractile response to tyramine is mediated via catecholamine release. The concentrations of cocaine used in this study markedly potentiated the contractile response to NA and inhibited the uptake of NA-^{3}H. A major difference between cocaine and the antidepressants is that cocaine has no alpha blocking activity whereas the antidepressants have (Brodie, Dick, Kielholz, Poldinger & Theobald, 1961 ; Ursillo & Jacobson, 1965 ; Osborne & Sigg, 1960, Trendelenburg, 1963). The alpha blocking activity of imipramine and amitriptyline can be inferred in this preparation because imipramine did not potentiate the contractile response to NA in a concentration (10^{-7}M) which significantly reduced the uptake of NA-^{3}H. A higher concentration of imipramine (10^{-6}M) actually shifted the NA curve to the right, as did amitriptyline 10^{-6}M. Thus alpha blockade was probably masking the possible potentiation due to uptake inhibition for both imipramine and amitriptyline. Our findings with DMI confirmed those of Ursillo & Jacobson (1965) in that the maximum potentiation reached with DMI was less than that reached with cocaine, presumably indicating that the potentiation of NA by DMI is limited by its alpha blocking property. This is further substantiated by the greater effect of DMI on NA-^{3}H uptake compared with cocaine (Table 1). Additional evidence for the hypothesis that alpha blockade may be the most important factor of tyramine antagonism in rat vas deferens is that cocaine antagonizes the pressor effects of

TABLE 1. *Inhibition of* in vitro *uptake of noradrenaline-^{3}H and its correlations with changes in contractile response to noradrenaline in rat vas deferens*

Drug	Concentration (M)	% inhibition (mean±S.E.) NA-^{3}H uptake*	Mean change in contractile response to NA ED50†
Cocaine	10^{-5}	44·4±0·8	+43%
Dexchlorpheniramine	10^{-5}	22·2±4·5	+29%
Imipramine	10^{-7}	33·1±3·8	−6%
Desmethylimipramine	10^{-7}	52·8±0·9	+32%

* Mean of four animals for each drug. † Mean of three animals for each drug.

tyramine competitively (Trendelenburg, 1961; Bonaccorsi & Garattini, 1966), whereas in rat vas deferens neither the antidepressants nor dexchlorpheniramine shifted the tyramine curve to the right. Phentolamine, as previously indicated, also non-competitively antagonizes the contractile response to tyramine. This latter effect seems paradoxical because phentolamine competitively antagonizes the contractile response to NA. If it is assumed that concentrations of tyramine higher than that needed to produce maximum contraction do not release more NA than the ED100, however, then raising the concentration of tyramine in the presence of phentolamine cannot overcome the alpha receptor blockade and reach the original maximum response.

The non-parallel shift to the left of the NA dose-response curve produced by dexchlorpheniramine is difficult to explain. Iversen (1965) has postulated that there are two concentration dependent uptake processes for NA in rat heart. An attractive hypothesis would be that dexchlorpheniramine only affects the low-concentration uptake system ($uptake_1$) and therefore does not shift the whole NA curve to the left. Cocaine reduced both $uptake_1$ and $uptake_2$ of NA in rat heart, but its effect on $uptake_1$ was much greater than on $uptake_2$. As indicated previously (Table 1), dexchlorpheniramine significantly reduced the uptake of NA-^{3}H in rat vas deferens. There is some evidence to support the hypothesis that dexchlorpheniramine antagonizes tyramine by alpha blockade. Antihistamines such as phenindamine, cyproheptadine and promethazine are known to have alpha blocking properties (Stone, Wenger, Ludden, Stavorski & Ross, 1961; Roth & Tabachnick, 1965), but these properties have not been demonstrated with dexchlorpheniramine. Dexchlorpheniramine, however, does inhibit tyramine non-competitively as does phentolamine, and the potentiation of NA by dexchlorpheniramine does reach a maximum which is substantially less than that produced by cocaine. The most convincing experiment would be to see if dexchlorpheniramine would antagonize NA in denervated rat vas deferens, a preparation in which uptake of NA is no longer a factor. Sectioning of the hypogastric nerve, however, which is the major nerve supply to the vas deferens, does not lead to complete adrenergic nerve degeneration in cats and guinea-pigs (Jacobowitz & Koelle, 1965). Future experiments will be devoted to studying whether the NA potentiating effects of dexchlorpheniramine are limited by alpha blockade or by inability completely to block NA uptake.

If drugs such as cocaine do not reduce the tyramine-induced release of NA, this is strong evidence to indicate that alpha adrenergic blockade is an important mechanism for tyramine antagonism. Experiments are in progress to investigate whether cocaine, imipramine, or dexchlorpheniramine can antagonize tyramine-induced release of NA-^{3}H from rat vas deferens *in vitro*.

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