

## **The effects of some $\beta$ -adrenoreceptor blocking drugs on the uptake and release of noradrenaline by the heart**

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1. The potencies of some  $\beta$ -adrenoreceptor blocking drugs in reducing noradrenaline uptake by the isolated heart were compared with their potencies in reducing the release of noradrenaline from the heart by tyramine.
  2. Of the drugs tested, propranolol, pronethalol and dichloroisoprenaline were the most potent in blocking uptake and release of noradrenaline, although none was as potent as cocaine ; MJ 1999 and I.C.I. 50172 were only weakly effective.
  3. Pronethalol and dichloroisoprenaline each reduced release of noradrenaline by tyramine in the concentration range ( $10^{-7}$ - $10^{-6}$ M) where blockade of responses to tyramine was apparent ; with these drugs both reduction of noradrenaline release and  $\beta$ -receptor blockade contribute to the reduction in responses to tyramine.
  4. Potency of  $\beta$ -receptor blocking drugs in reducing noradrenaline uptake is unrelated to potency in blocking  $\beta$ -receptors ; Kö 592 blocks  $\beta$ -receptors without affecting noradrenaline uptake.
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The first explanation for the blockade of the actions of tyramine by cocaine was offered by Macmillan in 1959. He suggested that cocaine, as well as preventing uptake of noradrenaline, also prevented release of noradrenaline from the tissue stores by tyramine. Many of the  $\beta$ -adrenoreceptor blocking drugs, including pronethalol, propranolol and dichloroisoprenaline, have been shown to act like cocaine in decreasing the uptake of noradrenaline by the heart and other tissues (Farrant, Harvey & Pennefather, 1964 ; Lindmar & Muscholl, 1964 ; Iversen, 1965 ; von Euler, 1966). This raises the possibility that there may be two components in the action of these drugs in preventing the action of tyramine on the heart ; they may reduce the release of noradrenaline by tyramine, in addition to antagonizing the effects of the released noradrenaline. In order to analyse the relative importance of these two components of action of some of the  $\beta$ -receptor blocking drugs, we have compared the potencies of dichloroisoprenaline, pronethalol, propranolol, (+)-propranolol, Kö 592, Ciba 39'089-Ba, MJ 1999 and I.C.I. 50172 in reducing uptake of noradrenaline by the isolated heart, and in preventing the actions of tyramine on the heart, with their potencies in blocking  $\beta$ -receptors.

## Methods

Hearts isolated from rabbits weighing 1.5–2.2 kg were perfused with McEwen's (1956) solution at a constant rate of 6 ml./min. The perfusion fluid was maintained at  $37^{\circ} \pm 0.1^{\circ}$  C and gassed with 5% carbon dioxide in oxygen. In some experiments, contractions of the heart were measured with a Statham transducer and the rate of beating with a Beckman Offner cardiometer (type 9857).

### *Uptake of noradrenaline*

Noradrenaline uptake by the isolated heart was measured by injecting noradrenaline (0.5  $\mu$ g) into the perfusion fluid, and assaying the noradrenaline appearing in the effluent during the 4 min period after the injection. The effluent was collected into a beaker containing sufficient HCl to make the final pH between 4 and 5. The difference between the amount of noradrenaline injected and the amount recovered represented uptake of noradrenaline by the heart. This method has been described to the British Pharmacological Society (Jowett & Stafford, 1965). Noradrenaline was injected at 20 min intervals; after two injections, the hearts were perfused with cocaine or one of the  $\beta$ -adrenoreceptor blocking drugs for 15 min before further determinations of uptake. Each heart was exposed to four concentrations of the drug under investigation; the lowest concentration tested was  $8 \times 10^{-8}$ M for cocaine and  $4 \times 10^{-7}$ M for each of the  $\beta$ -adrenoreceptor blocking drugs, and the concentration was increased 5-fold between each estimate of uptake. The results are expressed as percentage reduction in noradrenaline uptake, and each is the mean value from three experiments.

### *Release of noradrenaline by tyramine*

Injections of tyramine (250  $\mu$ g) were given at 20 min intervals into the perfusion fluid supplying the heart. After two injections of tyramine, cocaine or one of the  $\beta$ -adrenoreceptor blocking drugs was added to the perfusion fluid as described above. For 7–8 min after each injection of tyramine, the effluent from the heart was collected and acidified as above. The noradrenaline in the effluent was adsorbed on to approximately 0.5 g of alumina (Woelm) in the presence of sodium edetate (100 mg) at a pH range of 8.2–8.6 (Anton & Sayre, 1962). The noradrenaline was eluted from the alumina by extractions with aliquots of 0.2 N-HCl to make the final volume 5.0 ml. The pH of the eluate was adjusted with solid  $\text{NaHCO}_3$  to be between 4 and 5, and its noradrenaline content was estimated by bioassay. The mean recovery of 50 or 100 ng of noradrenaline added to 45 ml. of effluent from the heart, either with or without 250  $\mu$ g of tyramine, was  $87.3 \pm 3.6\%$  (S.E. of fifteen observations).

### *Bioassay of noradrenaline*

Noradrenaline was assayed either on the blood pressure of the pithed rat (Shipley & Tilden, 1947) or on the isolated central artery from the rabbit's ear (de la Lande & Harvey, 1965). To sensitize the rats to noradrenaline, they were injected intraperitoneally with reserpine (2.5 mg) 1 or 2 days before use, and with guanethidine (3–5 mg) immediately before the assay. The isolated artery was sensitized to noradrenaline by perfusion with 5-HT (40 ng/ml.) as described by de la Lande & Harvey (1965). In our hands, the pithed rat gave more reproducible results, and was used for most of the assays.

*Antiadrenaline activity*

Atria isolated from rabbits were suspended in McEwen's solution at  $30.5^{\circ} \pm 0.5^{\circ}$  C, and the rate of beating was recorded with a Thorp impulse counter (C. F. Palmer, London). The response to noradrenaline was measured as the increase in number of contractions during the 3 min after its injection into the bath.  $pA_2$  values for the antiadrenaline drugs were determined as described by Schild (1947) after a contact time of 15 min.

**Results***Reduction of noradrenaline uptake*

After single injections of 500 ng of noradrenaline into isolated hearts, the mean amount of noradrenaline recovered in the effluent was  $181 \pm 6$  ng (twenty-seven experiments). If it is assumed (see **Discussion**) that all the noradrenaline that does not appear in the effluent has been taken up by the heart, the mean uptake of noradrenaline was  $319 \pm 6$  ng, or 64% of the injected dose. The mean weight of the hearts at the end of each experiment was  $5.57 \pm 0.18$  g; there was no significant correlation between the uptake of noradrenaline and heart weight. Uptake remained constant for at least five subsequent injections of noradrenaline given at 20 min intervals.

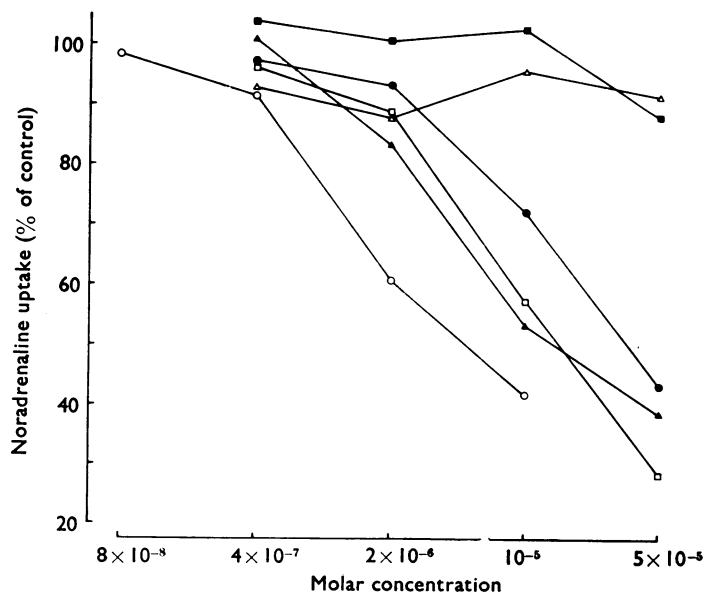


FIG. 1. Effects of cocaine and some  $\beta$ -adrenoreceptor blocking drugs on noradrenaline uptake by the isolated heart. Noradrenaline uptake was estimated by injecting noradrenaline (500 ng) at 20 min intervals, and assaying the amount recovered in the effluent. Between successive estimates of uptake, each heart was exposed to one of the following drugs at the concentrations indicated above: ■—■, I.C.I. 50172; △—△, MJ 1999; ●—●, propranolol; ▲—▲, dichloroisoprenaline; □—□, pronethalol; ○—○, cocaine. Each point is the mean result from three experiments.

Cocaine ( $8 \times 10^{-8} \text{M}$ ) reduced the uptake of noradrenaline by the hearts; with increasing concentrations of cocaine (up to  $10^{-5} \text{M}$ ), uptake was further reduced (Fig. 1). Each of the  $\beta$ -adrenoreceptor blocking drugs was tested in concentrations ranging from  $4 \times 10^{-7}$  to  $5 \times 10^{-5} \text{M}$ , for its action in blocking noradrenaline uptake. The mean results of experiments with pronethalol, propranolol, dichloroisoprenaline, MJ 1999, and I.C.I. 50172 are shown in Fig. 1. None of these drugs was as potent as cocaine in blocking uptake of noradrenaline by the isolated heart. Pronethalol, propranolol and dichloroisoprenaline were the most potent of the  $\beta$ -adrenoreceptor blocking drugs; MJ 1999 and I.C.I. 50172 were only weakly effective, even in a concentration as high as  $5 \times 10^{-5} \text{M}$ . The effects of Kö 592, Ciba 39'089-Ba and (+)-propranolol on noradrenaline uptake were also measured and these results are included in Table 1.

#### *Reduction of noradrenaline release by tyramine*

The amount of noradrenaline released from isolated hearts by tyramine ( $250 \mu\text{g}$ ) was variable, ranging from 34 to 200 ng. In twenty-one experiments, the mean release was  $84.0 \pm 8.8$  ng of noradrenaline, and there was no significant correlation between the amount of noradrenaline released and the heart weight. With subsequent injections of tyramine, the release of noradrenaline decreased slightly with each injection. After the sixth injection of tyramine, the mean amount of noradrenaline released was 89% of the initial value.

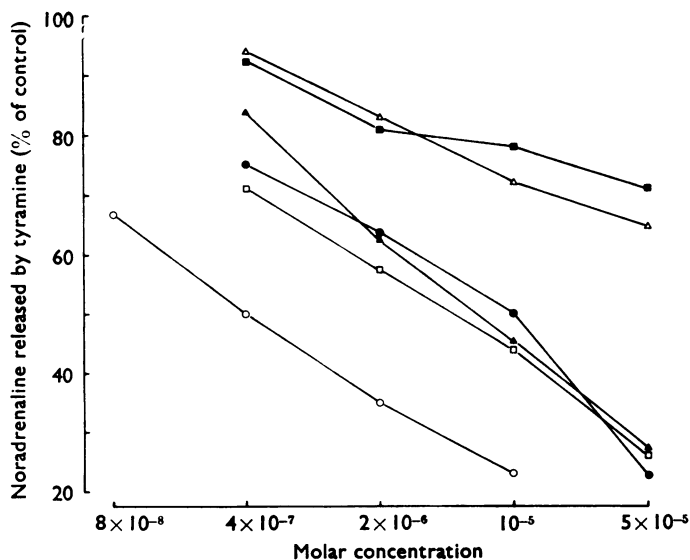


FIG. 2. Effects of cocaine and some  $\beta$ -adrenoreceptor blocking drugs on the release of noradrenaline from the isolated heart by tyramine. Injections of tyramine ( $250 \mu\text{g}$ ) were given at 20 min intervals. Between injections of tyramine, each heart was exposed to one of the following drugs at the concentrations indicated above: ■—■, I.C.I. 50172; △—△, MJ 1999; ●—●, propranolol; ▲—▲, dichloroisoprenaline; □—□, pronethalol; ○—○, cocaine. Each point is the mean result from three experiments.

In the presence of cocaine ( $8 \times 10^{-8}$ – $10^{-5}$ M), release of noradrenaline by tyramine was greatly reduced (Fig. 2). Pronethalol, propranolol and dichloroisoprenaline ( $4 \times 10^{-7}$ – $5 \times 10^{-5}$ M) also reduced the release of noradrenaline by tyramine, but none of these was as potent as cocaine (Fig. 2). MJ 1999 and I.C.I. 50172, shown to be the least effective in blocking noradrenaline uptake, were also the least effective in reducing noradrenaline release by tyramine. Comparison of Fig. 1 and 2 shows that those drugs that are more effective in reducing noradrenaline uptake are also more effective in reducing noradrenaline release by tyramine.

#### Relative potencies in blocking noradrenaline action

The relative potencies of eight  $\beta$ -adrenoreceptor blocking drugs in antagonizing the chronotropic action of noradrenaline on isolated atria are given in Table 1, which also shows their effectiveness in preventing noradrenaline uptake by the isolated heart. All the drugs tested blocked  $\beta$ -receptors in concentrations lower than those required to block noradrenaline uptake. There was no correlation between their potencies in blocking  $\beta$ -receptors and their potencies in blocking noradrenaline uptake.

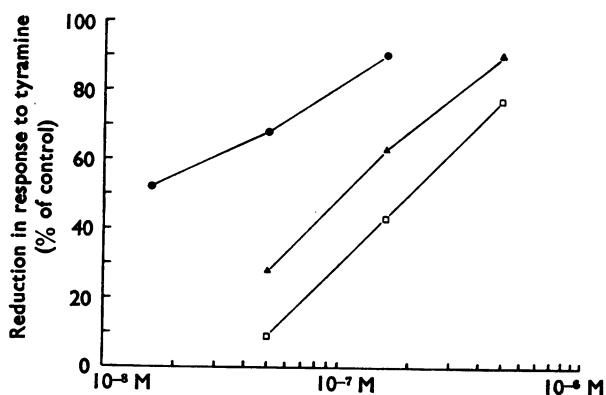


FIG. 3. Blockade of chronotropic responses of the isolated heart to tyramine by propranolol (●—●), dichloroisoprenaline (▲—▲) and pronethalol (□—□). Each point is the mean result from three experiments.

TABLE 1. Comparison of the relative potencies of  $\beta$ -adrenoreceptor blocking drugs with their effectiveness in blocking noradrenaline uptake

Drug	$pA_2$	Percentage inhibition of noradrenaline uptake*
I.C.I. 50172	6.4	0
Kö 592	7.7	2
MJ 1999	6.1	4
Ciba 39'089-Ba	7.9	10
(±)-Propranolol	8.2	27
(+)-Propranolol	6.1	31
Pronethalol	6.8	43
Dichloroisoprenaline	7.3	47

\* Each drug was used in the concentration of  $10^{-5}$ M.

### *Relative potencies in blocking tyramine action*

*Isolated hearts.* Because antagonism of responses to tyramine by pronethalol and propranolol is known to be noncompetitive (Benfey & Varma, 1964), no attempt was made to determine  $pA_2$  values for antagonism. Instead, we measured the percentage reductions in the chronotropic response to single doses of tyramine (25  $\mu\text{g}$ ) by increasing concentrations of pronethalol, propranolol and dichloroisoprenaline, drugs which were approximately equipotent in blocking noradrenaline release by tyramine (Fig. 2). After two control responses to tyramine, the hearts were perfused for 15 min with dichloroisoprenaline, pronethalol ( $5 \times 10^{-7}$ – $5 \times 10^{-6}\text{M}$ ) or propranolol ( $1.6 \times 10^{-8}$ – $1.6 \times 10^{-7}\text{M}$ ) before and during subsequent injections of tyramine. The mean results of three experiments with each of these drugs are shown in Fig. 3, from which it can be seen that propranolol was the most potent in antagonizing the acceleration of the heart rate produced by tyramine, and pronethalol the least potent. The rank order of potency of these drugs in antagonizing tyramine and in antagonizing noradrenaline is the same, suggesting that the action on  $\beta$ -receptors is an important factor in their antagonism of tyramine. Propranolol effectively suppressed the responses to tyramine in doses lower than those appreciably reducing noradrenaline release by tyramine, and it therefore appears that  $\beta$ -receptor blockade accounts for most of its antagonistic action. With dichloroisoprenaline and pronethalol, however, reduction of noradrenaline release may be contributing to the blockade of the action of tyramine.

### **Discussion**

This method of measuring noradrenaline uptake into the isolated heart by estimating overflow after a single injection into the perfusion fluid has not been used before to compare the relative potencies of drugs that interfere with noradrenaline uptake. The uptake process involved probably corresponds to that designated Uptake<sub>1</sub> by Iversen (1967), because noradrenaline is more readily taken up than adrenaline, and isoprenaline is not taken up at all (Jowett & Stafford, 1965); furthermore, the concentration of noradrenaline in the perfusion fluid reaching the heart after a single injection of 500 ng would not be high enough for the Uptake<sub>2</sub> process to be important. This method has the advantage that many successive estimations of noradrenaline uptake can be made using the same heart, which can be exposed to several concentrations of drugs that affect uptake. It has the disadvantage that there is no direct measurement of the amount of noradrenaline taken up, and metabolism or decomposition of noradrenaline may contribute to the loss observed. After perfusion of the heart with high concentrations of cocaine, however, more than 90% of the injected noradrenaline was recovered in the effluent, and this suggests that most of the difference between injected and recovered noradrenaline is due to uptake by the heart, and that inactivation by other processes is probably slight. The results obtained with this method are in reasonable agreement with those for cocaine, dichloroisoprenaline and pronethalol as described by Iversen (1965) using the isolated rat heart, although the degree of inhibition of uptake in our experiments was consistently somewhat less. This may reflect a real species difference in the sensitivity of the uptake process to inhibition by drugs, or it may mean that our method gives an underestimate of the degree of inhibition.

The order of potency of pronethalol, propranolol, dichloroisoprenaline, MJ 1999 and I.C.I. 50172 in blocking uptake of noradrenaline by the heart was related to

their potency in reducing the release of noradrenaline by tyramine. It is clear from these results that some of these drugs could block the action of tyramine both by blocking  $\beta$ -receptors and by reducing the release of noradrenaline by tyramine.

Drugs that block noradrenaline release by tyramine would be expected to block its vasoconstrictor action as well as its action on the heart. In general, the amounts of the  $\beta$ -receptor blocking drugs required to block vasoconstrictor responses would be appreciably higher than those required to block  $\beta$ -receptors. Aramendia & Kaumann (1967), however, described blockade of vasoconstrictor responses to tyramine and to sympathetic stimulation by MJ 1999 in the perfused hind limb of the dog. That MJ 1999 can reduce release of noradrenaline by tyramine is shown in Fig. 2, but the concentrations required to produce this effect are at least 25 times greater than those of dichloroisoprenaline, pronethalol and propranolol. Westfall (1967) found that MJ 1999 (10 mg/kg) did not block uptake of noradrenaline by the rat heart *in situ*.

There is no correlation between the potencies of these drugs in blocking  $\beta$ -receptors and in blocking uptake of noradrenaline. Kö 592 has a powerful  $\beta$ -receptor blocking action with no appreciable effect on noradrenaline uptake, and it would therefore be the most suitable to use if a selective action were required.

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