Br. J. Pharmac. Chemother. (1966), **27**, 506–513.

EFFECT OF INFUSION OF METARAMINOL ON THE RESPONSE OF RESERPINE-PRETREATED SPINAL CATS TO TYRAMINE AND TO NORADRENALINE

BY

B. BHAGAT AND R. RAGLAND

From the Department of Pharmacology, College of Medicine, Howard University, Washington, D.C.

(Received June 3, 1966)

While noradrenaline is a neurochemical transmitter of the sympathetic nervous system in mammals (Euler, 1956) a number of sympathomimetic amines, chemically related to noradrenaline, have been shown to behave like this catecholamine. Following administration, these amines such as α-methyl-noradrenaline, metaraminol, etc., are taken up by sympathetic nerve endings and may replace the noradrenaline from its storage site(s). These amines can be released by sympathetic nerve stimulation and by reserpine, guanethidine and tyramine, all of which deplete noradrenaline. The uptake of these amines is inhibited by drugs inhibiting noradrenaline uptake. Thus these amines act as " false neurotransmitters " (Kopin, 1966).

The pressor action of tyramine, shown by Carlsson, Rosengren, Bertler & Nilsson (1957) to be absent in animals pretreated with reserpine, is restored by infusion of noradrenaline (Burn & Rand, 1958). This restoration is believed to be due to a refilling by exogenous noradrenaline of the previously depleted stores and a subsequent release by tyramine of the stored noradrenaline. However, this restoration is not confined to noradrenaline, since metaraminol and other false neurotransmitters have been shown recently to restore the pressor action of tyramine (Day & Rand, 1964; Bhagat, Bovell & Ragland, 1966).

The aim of the present study was to investigate the influence of graded doses of metaraminol on restoration of the pressor response to tyramine in reserpine-pretreated cats. Since the response to tyramine also depends on the sensitivity of the receptors of the effector organs to noradrenaline, responsiveness to noradrenaline, after metaraminol administration, was also investigated.

METHODS

Forty-three cats of either sex, weighing between 1.8 and 2.2 kg, were anaesthetized with ether and spinal preparations were set up as described by Burn (1952). The movements of the nictitating membrane were recorded isometrically with a transducer (myograph-B); the tension on the nictitating membrane was 5 *g*. The blood pressure was recorded from the femoral artery with a blood pressure tranducer (Linear-Core model P-1000). The transducers were connected with a Physiograph. Intra-

venous infusions were made into the left femoral vein over a 30-min period. Intravenous injections were made into the right femoral vein and flushed in with 0.5 ml. of 0.9% saline. Reserpine (1 mg/kg) was given intraperitoneally 24 hr prior to the experiment. The response to tyramine was tested 20 min before and 45 min after the infusion of various doses of metaraminol.

The following substances were used: L-metaraminol bitartrate, L-noradrenaline bitartrate monohydrate, reserpine phosphate (the doses refer to the bases); tyramine hydrochloride and cocaine hydrochloride (the doses refer to the salts). The details of dosage and routes of administration are given in Results.

RESULTS

Restoration of the response to tyramine. The response of the nictitating membrane and of the blood pressure to the intravenous infusion of various doses of metaraminol was determined 20 min before and 45 min after infusion. To prevent the possibility of tachyphylaxis or exhaustion of the stores by repeated injections of tyramine, only one injection of this amine was given after the end of an infusion. Results (Fig. 1, 2)

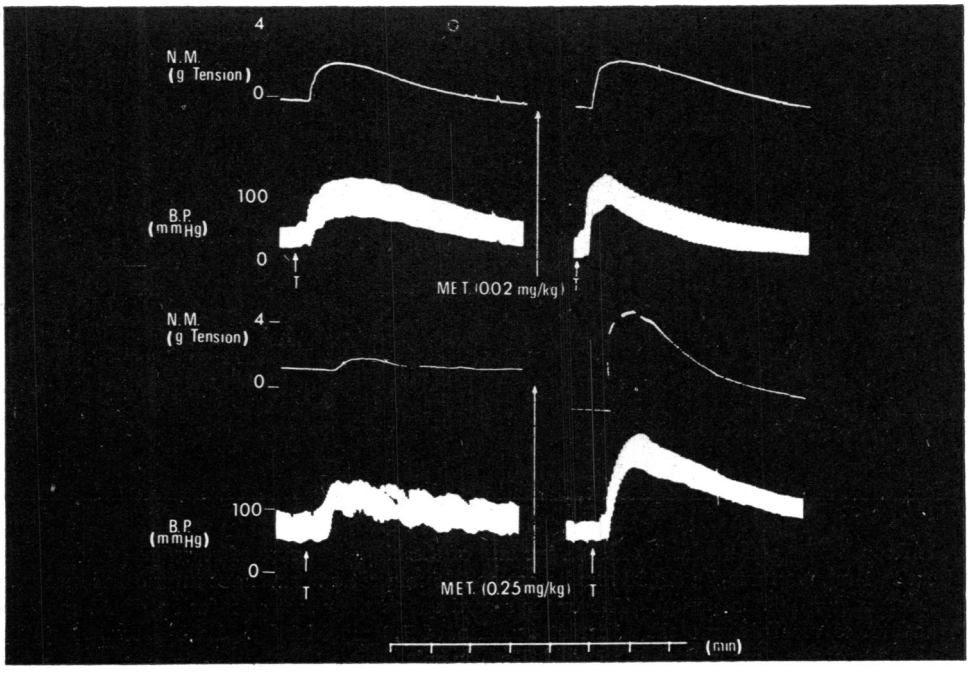

Fig. 1. Responses of the nictitating membrane and of the blood pressure of reserpine-pretreated spinal cat to 1 mg tyramine/kg (T) 20 min before and 45 min after intravenous infusion of metaraminol (Met) over a 20-min period. The upper record shows the effect of infusion of 0.020 mg Met/kg and lower record shows the effect of infusion of 0.25 mg Met/kg. Each record is typical of four experiments. Time in min.

show, that the ability of metaraminol to enhance or restore the response to tyramine was dose-dependent. While low doses of metaraminol (0.020 to 0.25 mg/kg) restored the pressor response to tyramine, high doses (0.5 to 1 mg/kg) failed to do so; in fact a decrease in the response occurred.

The effect of an infusion of metaraminol on the response of the nictitating membrane did not follow the same pattern. A dose of 0.020 mg/kg metaraminol slightly enhanced the response to tyramine (Fig. 1) and a dose of 0.25 to 5.0 mg/kg enhanced the response significantly (Fig. 1, 2) while a dose of 1 mg/kg metaraminol failed to affect it (Fig. 2). Since the nictitating membrane did not relax after an infusion of 1 mg/kg metaraminol, the reduced magnitude of the response to tyramine may be the consequence of the remaining contraction.

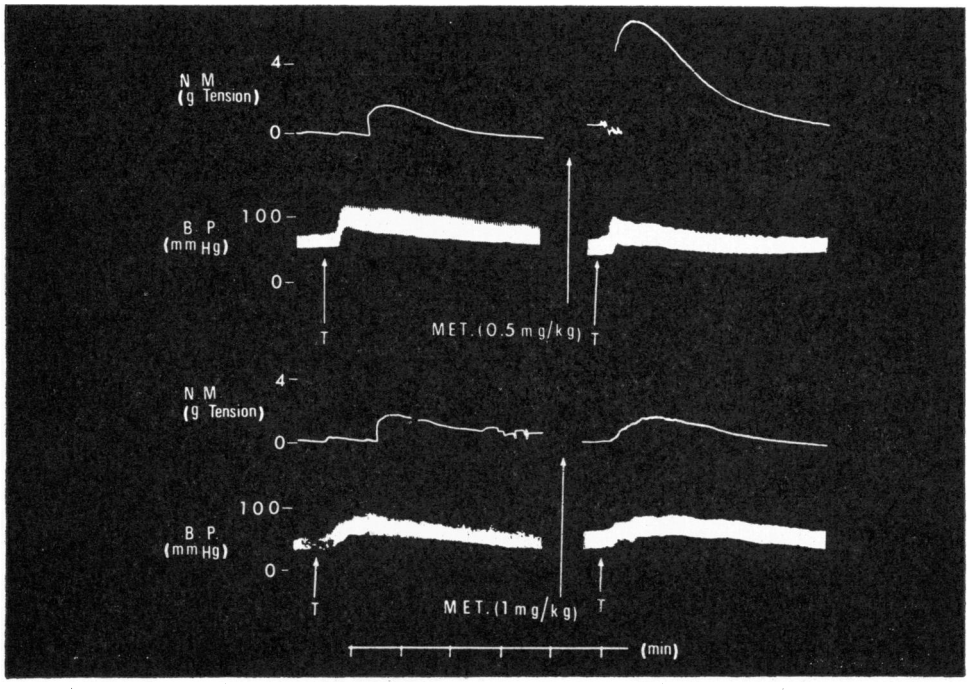

Fig. 2. Responses of the nictitating membrane and of the blood pressure of reserpine-pretreated spinal cat to 1 mg tyramine/kg (T) 20 min before and 45 min after intravenous infusion of metaraminol (Met) over a 20-min period. The upper record shows the effect of infusion of 0.5 mg Met/kg and lower record shows the effect of infusion of 1.0 mg Met/kg. Each record is typical of six and four experiments respectively. Time in min.

Sensitivity of the blood pressure to noradrenaline in reserpine-pretreated spinal cats after infusion of metaraminol. Since the nictitating membrane did not relax completely and maintained a high tone after certain doses of metaraminol, it was difficult to compare

the response of the nictitating membrane to noradrenaline in various preparations ; values are therefore not presented. Dose-response curves for noradrenaline on the blood pressure were obtained 10 min before and 45 min after an infusion of metaraminol. Four to six animals were used in order to obtain the dose-response curves for noradrenaline for each dose-level of metaraminol. To measure the sensitivity to noradrenaline after metaraminol, the log dose of noradrenaline was plotted against percent of the maximum response. From each individual dose-response curve, a dose which caused 70% of the

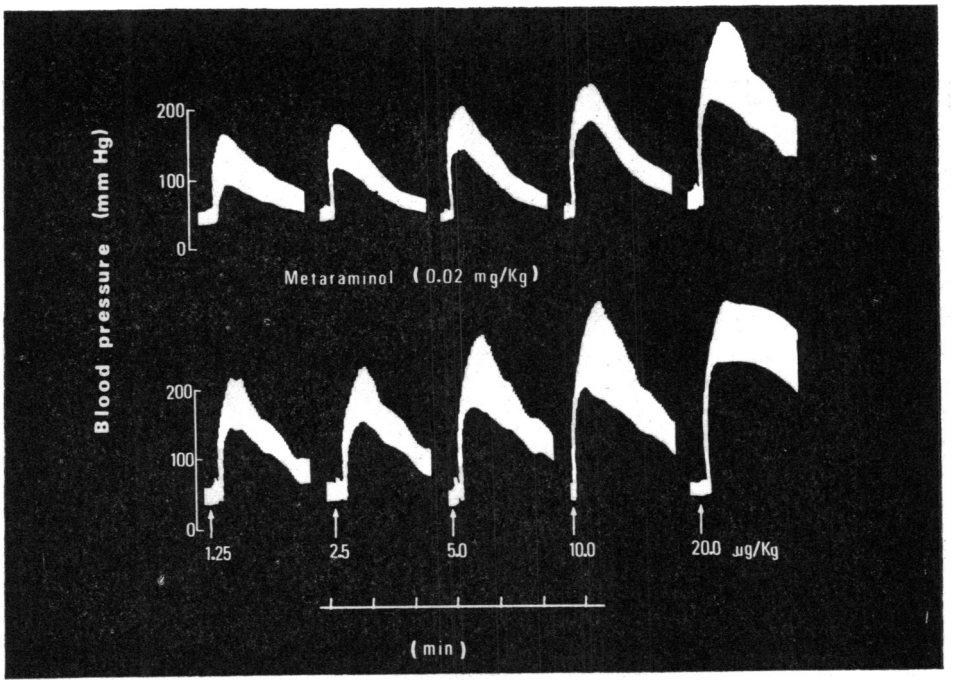

Fig. 3. Response of the blood pressure of reserpine-pretreated spinal cat (2.7 kg) to various doses of noradrenaline before and after infusion of metaraminol (0.020 mg/kg) over a 20-min period. The upper trace before and lower trace 45 min after infusion of metaraminol. Each record is typical of four experiments. Time in min.

maximum response was calculated ; this dose is referred to as ED70 in the text. The ratio ED70 before over ED70 after metaraminol is a measure of sensitivity developed by individual preparations after metaraminol.

The ED70 for noradrenaline obtained for normal animals was 1.8 ± 0.4 µg/kg (mean $\pm$S.E. of six experiments). Pretreatment of animals with reserpine 24 hr prior to the experiment caused no shift of the dose-response curve. The ED70 for such animals

was 2.11 ± 0.3 μg/kg (mean $\pm$ S.E. of twelve experiments). The difference between the two means is not statistically significant ($P > 0.05$).

Metaraminol at a dose of 0.020 mg/kg caused a potentiation of the response to noradrenaline (Fig. 3) and therefore shifted the dose-response curve to the left. The ratio ED70 before over ED70 after this dose of metaraminol is 2.4 ± 0.4 (four experiments).

At a dose level (0.5 mg metaraminol/kg) there was no significant shift of the dose-response curve (Fig. 4). The ratio ED70 before over ED70 after metaraminol (0.5 mg/kg) is 0.8 ± 0.1 (six experiments).

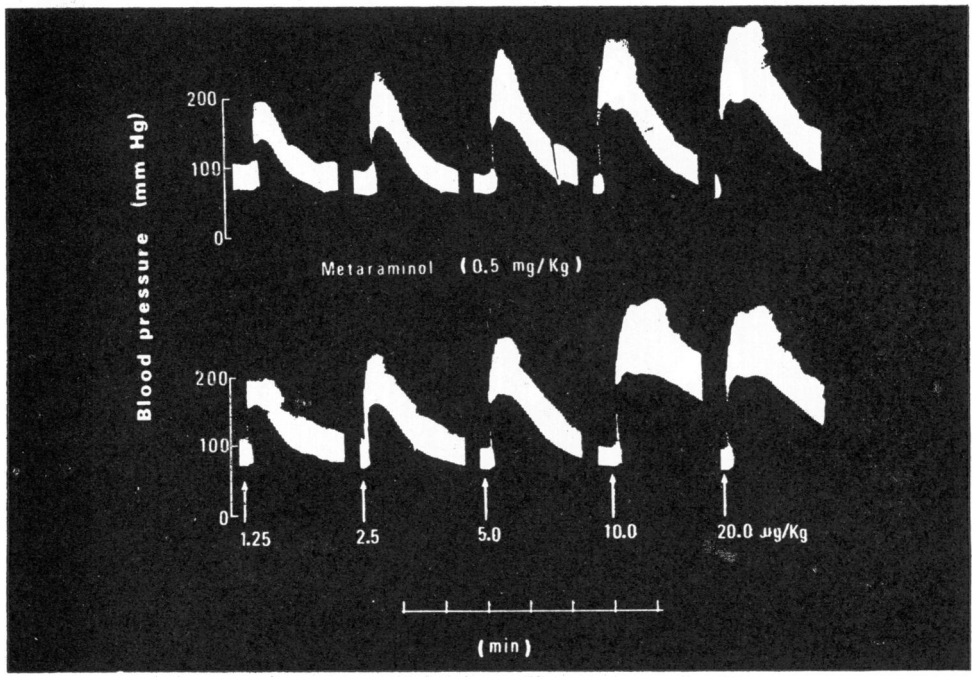

Fig. 4. The response of the blood pressure of reserpine-pretreated spinal cat (2.4 kg) to various doses of noradrenaline before and after infusion of metaraminol (0.5 mg/kg) over a 20-min period. The upper trace before and lower trace 45 min after infusion of metaraminol. Each record is typical of six experiments. Time in min.

The dose of 1 mg metaraminol/kg caused subsensitivity to noradrenaline and therefore shifted the dose-response curve to the right (Fig. 5). The ratio ED70 before over ED70 after metaraminol is 0.11 ± 0.08 (six experiments). Administration of 5 to 10 mg/kg cocaine by a slow intravenous infusion failed to cause the usual potentiation to noradrenaline.

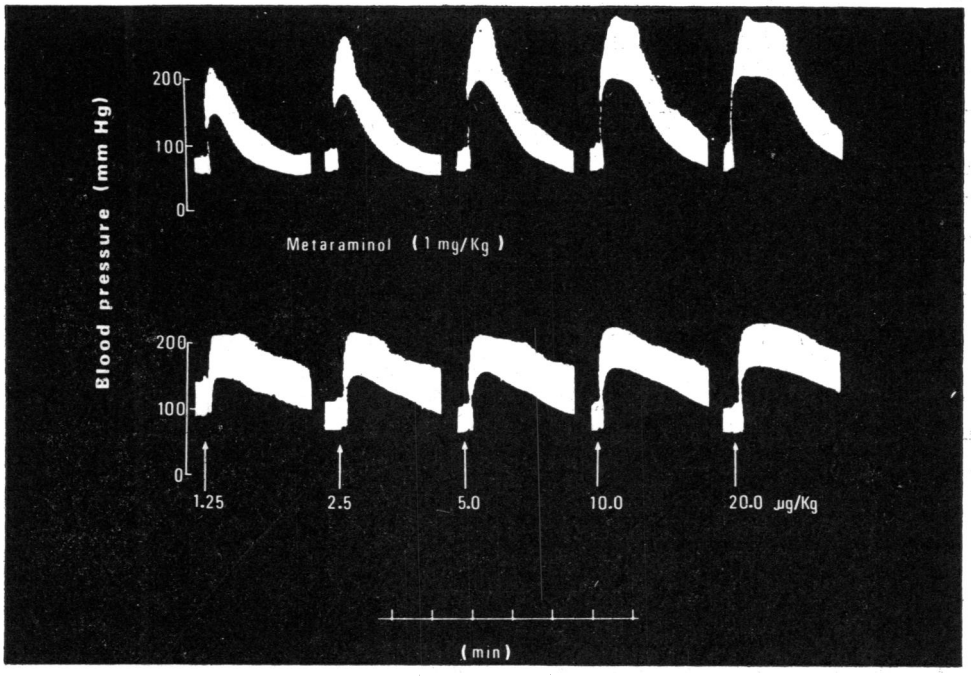

Fig. 5. The response of the blood pressure of reserpine-pretreated spinal cat (2.1 kg) to various doses of noradrenaline before and after infusion of metaraminol (1.0 mg/kg) over a period of 20 min. The upper trace before and lower trace 45 min after infusion of metaraminol. Each record is typical of six experiments. Time in min.

DISCUSSION

Pretreatment of animals with reserpine depletes their noradrenaline stores and thereby reduces the response of various organs to tyramine. However, the response to tyramine can be restored after an infusion of noradrenaline or its precursors (Burn & Rand, 1960; Trendelenburg & Pfeffer, 1964). This is generally interpreted to mean that exogenous noradrenaline is taken up by the tissue stores and subsequently released by tyramine. The restoration of the response to tyramine, however, is not restricted to noradrenaline, since unnatural amines like α-methyl noradrenaline or metaraminol are also capable of restoring the response to tyramine. Thus these amines following administration are bound in the nerve ending at sites ordinarily occupied by noradrenaline. The present study confirms that low doses of metaraminol (0.020 to 0.25 mg/kg) could restore the response to tyramine in reserpine-pretreated spinal cats. However, the restoration of the vasopressor response to tyramine was dose-dependent, since 0.5 mg/kg or 1 mg/kg metaraminol failed to restore the pressor response to tyramine; in fact the responses to tyramine after metaraminol were slightly decreased.

The response of any organ to tyramine depends on two factors at least: (a) the amount of neurotransmitters available for release and (b) the sensitivity of the organ to noradrenaline. The reduced sensitivity to noradrenaline observed after high doses of metaraminol may explain the diminished response to tyramine.

The effect of an infusion of metaraminol on the response of the nictitating membrane to tyramine was different from that of the blood pressure. A dose of 0.02 mg metaraminol/kg slightly enhanced the response to tyramine, doses of 0.25 to 0.5 mg/kg enhanced the response significantly, while a dose of 1 mg metaraminol/kg produced no change. Since the nictitating membrane did not relax after an infusion of 1 mg metaraminol/kg the reduced magnitude of the response to tyramine may be the consequence of the remaining contraction. Different efficacy at various receptors and perhaps organ differences in the inactivation of a false transmitter substance may be responsible for a lack of uniform reactivity of the sympathetic system.

Several investigators have demonstrated that circulating noradrenaline is readily taken up and bound in tissues more specifically by sympathetic nerve endings (Bhagat, 1963). This mode of inactivation seems to be more important in limiting the action of noradrenaline than its metabolic degradation. Compounds like cocaine, imipramine and chlorpromazine which interfere with the uptake of noradrenaline into the nerve terminals cause supersensitivity to noradrenaline, because noradrenaline may reach higher concentrations at the receptors of the effector organ. Metaraminol, like cocaine, inhibits the uptake of noradrenaline (Iversen, 1964) and, therefore, is expected to cause supersensitivity to this amine. The present study is consistent with this concept since low doses of metaraminol (0.02 mg/kg) caused increase of sensitivity to noradrenaline of the blood pressure.

The retention of exogenous noradrenaline must be regarded as a combination of two steps, actual uptake into the nerve terminal followed by storage (presumably in vesicles). Pretreatment with reserpine leaves the first step intact, but prevents the second (Lindmar & Muscholl, 1964). Thus, in reserpine pretreated animals, noradrenaline is transported at a normal rate, but instead of being stored in vesicles it is inactivated enzymatically by monoamine oxidase. This could explain why there is no supersensitivity to noradrenaline (as seen in the present study also) 24 hr after pretreatment with reserpine. Likewise, metaraminol, following its administration in reserpine pretreated animals, is taken up into nerve terminals (Shore, Busfield & Alpers, 1964), but is not inactivated either by binding (because of the reduced capacity of the organs to retain after reserpine) or by enzymatic destruction (because it is not a substrate for monoamine oxidase or catechol-*O*-methyl transferase). Therefore it is conceivable that high concentrations of metaraminol may be reached in the vicinity of the receptors of the effector organ. A direct consequence of this would be that many of the adrenergic receptors would be occupied by metaraminol. When noradrenaline is administered intravenously, it may occupy a fewer number of receptors. If Paton's rate theory is correct then it may explain the diminished response, i.e., subsensitivity to noradrenaline as observed in the present study after metaraminol (0.5 to 1 mg/kg). This would also explain why cocaine failed to exert its sensitizing effect in these preparations.

SUMMARY

1. The response of the nictitating membrane and of the blood pressure of reserpine-pretreated spinal cats to tyramine (1 mg/kg) or to various doses of noradrenaline was

determined before and after intravenous infusion of various concentrations of metaraminol.

2. Infusion of low doses (0.02 to 0.25 mg/kg) enhanced the response of the nictitating membrane and of the blood pressure to tyramine (1 mg/kg) in reserpine-pretreated spinal cats. Metaraminol at a dose of 0.5 mg/kg enhanced the response of the nictitating membrane but not that of the blood pressure to tyramine; while 1 mg/kg metaraminol failed to affect either of these responses.

3. A dose of 0.020 mg metaraminol/kg caused an increase in sensitivity of the blood pressure to noradrenaline; 0.5 mg metaraminol/kg did not alter the sensitivity; while 1 mg/kg caused subsensitivity. Cocaine (5 to 10 mg/kg) failed to cause the usual potentiation to noradrenaline after 1 mg metaraminol/kg.

4. Since metaraminol is resistant to destruction by monoamine oxidase because of its α-methyl group in the side chain, and since after reserpine the ability of the stores to retain amines is reduced, it is conceivable that, following administration of these drugs, a high concentration of metaraminol may build up in the vicinity of the receptors and occupy most of them, resulting in a decrease in sensitivity to noradrenaline and diminished response to tyramine.

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