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Modification of the vasoconstrictor action of sympathomimetic agents by bretylium tosylate and tranylcypromine in man

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1. The vasoconstrictor actions of tyramine, methylamphetamine and ephedrine on the blood vessels of the human hand have been found to be potentiated by administration intra-arterially of the adrenergic neurone blocking agent, bretylium tosylate.

2. One mechanism suggested for the enhancement of vasoconstriction is that bretylium possesses monoamine oxidase inhibiting activity, which, in the case of tyramine, is protective both to the sympathomimetic agent and the intraneuronal transmitter which it releases. In the case of methylamphetamine and ephedrine, which are not substrates for the enzyme, protection of the intraneuronal transmitter alone might occur, accounting for the lesser degree of potentiation of the effect of these amines by bretylium.

3. Comparison of the influences of bretylium and the monoamine oxidase inhibitor, tranylcypromine, on the vasoconstrictor action of the sympathomimetic agents shows a similar pattern of enhancement in the presence of both these drugs.

4. Tranylcypromine caused enhancement of the response of the hand vessels to noradrenaline, and this action could contribute to its potentiation of the effect of the sympathomimetic amines.

5. For a monoamine oxidase inhibiting action of bretylium to be effective in potentiating the constrictor actions of the sympathomimetic agents on the hand blood vessels at a time when reflex sympathetic activity is blocked it is necessary to postulate that these drugs and reflex nerve activity act either on different compartments of the transmitter store or by different release mechanisms.

Bretylium tosylate is a hypotensive agent whose mechanism of action is not fully understood. Its principal effect is blockade of the sympathetic adrenergic neurones, an action considered to be due to the prevention of release of noradrenaline from the termination of the post-ganglionic fibres (Exley, 1960 ; Laurence, 1962). Green (1962) reported that the drug had an exceptionally high affinity for sympathetic ganglia and post-ganglionic nerve trunks, and McCoubrey (1962) found that it possessed antimonoamine oxidase activity, although this facet of its action was weak.

The vasoconstrictor actions of the sympathomimetic amines, methylamphetamine, ephedrine and tyramine, on human vessels have been shown to be entirely dependent on the presence of the sympathetic nerve endings (Parks, Sandison, Skinner & Whelan, 1961 ; Frewin & Whelan, 1968 a, b). The present paper reports the potentiation by bretylium of the constrictor action of these sympathomimetic amines on the hand blood vessels in man and compares this effect with a potentiating action on these drugs of the monoamine oxidase inhibitor, tranylcypromine.

Methods

The subjects for these experiments were normal volunteer medical students.

The experiments were carried out at laboratory temperatures ranging from 24° to 28° C, the subjects lying recumbent on a couch for at least 30 min before the observations were made, during which time the recording apparatus was applied and the infusion needle inserted.

Hand blood flow was measured by venous occlusion plethysmography, using water-filled plethysmographs maintained at a temperature of 32°–33° C (Greenfield, 1954), three or four records of flow being obtained each minute.

Intra-arterial drug infusions of 4 to 5 min duration were given into the brachial artery at the elbow of one side through a 22-gauge needle connected by a length of polyethylene tubing to a mechanically driven syringe which delivered 2 ml. of solution per min. Saline (0·9% w/v) was infused during the control periods and also used as a vehicle for the drugs. The doses of drugs were such that they did not produce systemic effects, making it possible to use the opposite uninfused limb as a control.

Percentage changes in hand flow produced by the sympathomimetic agents were determined from the averaged flow values during the 2 min before the drug infusion and the last 2 min of the infusion period, by which time the responses to the drugs had become stable. Allowance was made for spontaneous variations in the flow unrelated to drug action by assuming that in the absence of each drug infusion the infused and the control sides would have maintained the same relationship to each other as in the pre-infusion period (Duff, 1952).

The drugs used were tyramine hydrochloride (Koch-Light Laboratories Ltd), ephedrine hydrochloride (David G. Bull Laboratory Pty, Ltd), methylamphetamine hydrochloride (Methedrine, Burroughs Wellcome), bretylium tosylate (Darenthin, Burroughs Wellcome), tranylcypromine sulphate (Smith, Kline & French) and noradrenaline bitartrate monohydrate (Levophed, Winthrop). The doses of drugs are expressed as weights of their salts, except in the case of noradrenaline in which the weight of the base is used. Ascorbic acid (1 : 50,000) was added to the noradrenaline solutions.

The constrictor responses of the hand blood vessels to intra-arterial infusions of tyramine (50 or 75 μg/min), methylamphetamine (10 or 20 μg/min) and ephedrine (25 or 50 μg/min) were compared in five experiments with each amine 15–20 min before and 30–50 min after the administration of bretylium tosylate (4 mg/min for 5 min) and of tranylcypromine (50 μg/min for 5 min). The dose of sympathomimetic agent chosen for each subject was that expected to produce a fall in hand blood flow within the range of 20–50%.

Bretylium tosylate caused an initial constriction of the hand vessels in most of the

experiments. This persisted for about 5 minutes after the infusion ceased. The hand blood flow then gradually rose within the next 10 minutes to or slightly above the previous resting level (Cooper, Fewings, Hodge & Whelan, 1963).

To test for the sympathetic blockade of the hand blood vessels caused by the intra-arterial infusion of bretylium, ice was applied to the neck of the subject and the resulting vasoconstriction in both hands recorded. This procedure is a potent sympathetic stimulus and usually produces intense vasoconstriction in both hands (Cooper *et al.*, 1963). When the effects of bretylium were fully developed, the hand vessels on the treated side no longer constricted when ice was applied to the neck, while on the untreated side vasoconstriction of the same magnitude as before was seen. Approximately 30 min were required in most of the experiments before blockade of the hand blood vessels was fully effective, and the second infusion of each sympathomimetic was given 35–45 min after bretylium administration.

Tranylcypromine caused an initial constriction of the hand vessels which persisted throughout the infusion period. The blood flow returned to about the previous resting level approximately 15 min after the infusion ceased. A second infusion of each sympathomimetic agent was given 30–50 min after the tranylcypromine infusion, corresponding in time with those following bretylium.

The effect of tranylcypromine on hand vessel sensitivity was determined in five experiments in which noradrenaline (50 ng/min for 5 min) was given 10 min before and at 10 min intervals after tranylcypromine (50 μg/min for 5 min). The second and subsequent infusions of noradrenaline were given at a time when the blood flow had returned to the previous resting level. To conform with the time sequence

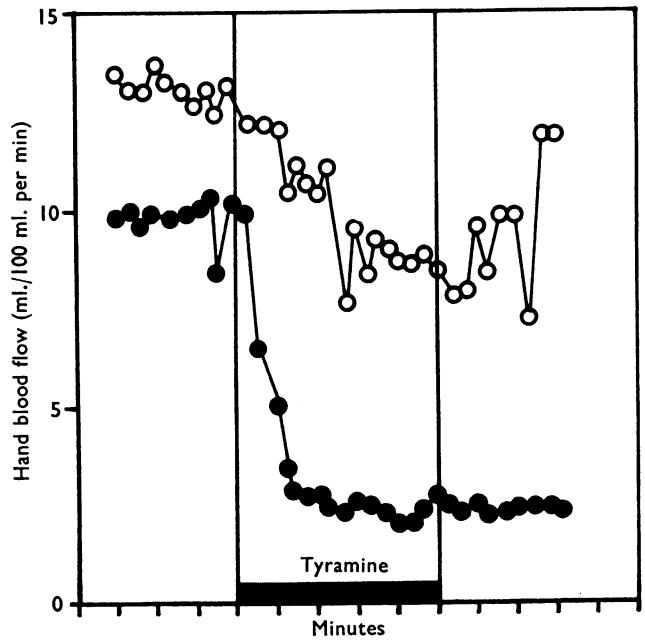

FIG. 1. Constrictor response of the hand blood vessels to intra-arterial infusion of tyramine (75 μg/min; black rectangle) 19 min before (○) and 40 min after (●) intra-arterial administration of bretylium tosylate (4 mg/min for 5 min).

which was observed in the experiments with the sympathomimetic amines, the percentage fall in flow caused by the noradrenaline infusion given 30–40 min after tranylcypromine was the one used in the calculations.

Results

The response of the blood flow through the hand to tyramine (75 μg/min for 5 min) before, and then after, bretylium tosylate (4 mg/min for 5 min) in one subject is shown in Fig. 1, both drugs being given by infusion into the brachial artery. The degree and duration of the constrictor response to tyramine was markedly enhanced after bretylium administration. Similar results were obtained in each of four other subjects, and Fig. 2A shows the falls in hand blood flow produced by tyramine in all five subjects expressed as percentage fall from the resting level of flow. The symbols to the left of the figure represent the values for percentage fall in flow caused by tyramine before treatment of the hand blood vessels with bretylium, and those to the right of the figure those after treatment. The enhancement of the vasoconstrictor action of tyramine averaged 41·4% following bretylium, an increase which was statistically significant ($0{\cdot}0005<P<0{\cdot}0025$).

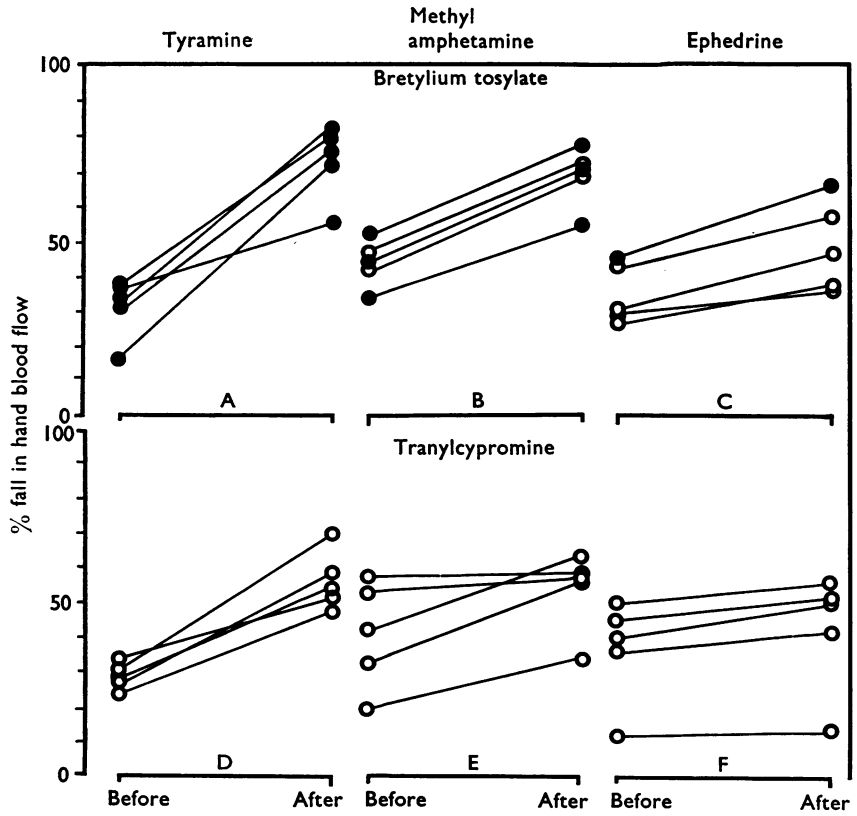

FIG. 2. Per cent fall in hand blood flow in response to tyramine (○, 50 μg/min; ●, 75 μg/min), methylamphetamine (○, 10 μg/min; ●, 20 μg/min) and ephedrine (○, 25 μg/min; ●, 50 μg/min) before and after bretylium tosylate (4 mg/min for 5 min) (A, B and C, respectively) and before and after tranylcypromine (50 μg/min for 5 min) (D, E and F, respectively). Five experiments, each on a different subject, were carried out with each sympathomimetic, the infusions being given 15–20 min before and 30–50 min after the administration of bretylium or tranylcypromine.

Figure 2B shows the results from five experiments on five subjects with methylamphetamine, the doses used being 20 μg/min on three occasions and 10 μg/min on two occasions. After treatment with bretylium the enhancement of the constrictor response of the hand blood vessels to methylamphetamine was not as well marked as with tyramine, being on average 24·6%, a significant difference ($P<0{\cdot}0005$).

Figure 2C shows the results obtained from five experiments on five subjects in which ephedrine was given at infusion rates of 25 μg/min (on four occasions) and 50 μg/min (on one occasion). A significant enhancement of the constrictor action to ephedrine was seen after bretylium treatment, averaging 12·16% ($0{\cdot}0025<P<0{\cdot}005$), which was smaller than that seen in the case of the other two sympathomimetic agents.

The lower three frames of Fig. 2 show the effects of the monoamine oxidase inhibitor, tranylcypromine, on the percentage falls in hand blood flow produced by tyramine, methylamphetamine and ephedrine, respectively. Figure 2D shows the results from five experiments on five subjects with tyramine (50 μg/min). In the presence of tranylcypromine the enhancement of the constrictor response of the hand blood vessels to tyramine averaged 27·5%, this being a significant increase ($0{\cdot}0005<P<0{\cdot}0025$).

Fig. 2E shows the results from five experiments on five subjects with methylamphetamine (10 μg/min), and Fig. 2F those from five experiments on five subjects with ephedrine (25μg/min). Tranylcypromine enhanced the constrictor response to methylamphetamine by 12·9% ($0{\cdot}0125<P<0{\cdot}025$), while the constrictor response to ephedrine was increased by an average of 5·9% ($0{\cdot}0025<P<0{\cdot}005$).

The means of the increases in percentage fall in hand blood flow caused by tyramine, methylamphetamine and ephedrine in the presence of bretylium and tranylcypromine are shown in Fig. 3. The trend in the enhancement of the constrictor responses to the sympathomimetic amines caused by both the hypotensive agent and the monoamine oxidase inhibitor is similar.

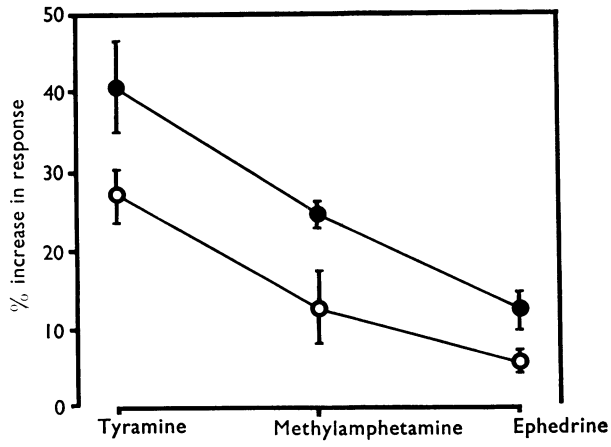

FIG. 3. Data in Fig. 2 averaged and expressed as % increase in response of the hand vessels to tyramine, methylamphetamine and ephedrine after bretylium (●) and after tranylcypromine (○) The vertical lines through each symbol represent one standard error on either side of the mean.

Effect of tranylcypromine on the response to noradrenaline

Five experiments were carried out in which noradrenaline (50 ng/min for 5 min) was given intra-arterially and the constrictor effect on the hand vessels recorded (Fig. 4). Tranylcypromine (50 μg/min for 5 min) was then infused over a 5 min period and later the noradrenaline infusion repeated. In every case the response to noradrenaline was enhanced by the prior administration of tranylcypromine, the mean % increase being 16·3% which was statistically significant ($0{\cdot}0005 < P < 0{\cdot}0025$).

Control experiments

Three experiments were carried out in which repeated infusions of the same dose of tyramine were made at intervals of 10 min, saline (0·9% NaCl) at a rate of 2 ml./min being continuously infused between the periods of drug administration. The constrictor responses were very reproducible and did not vary from the mean in each case by more than 6% (average 2·5%, 2·3% and 2·2%, respectively).

In four experiments the vehicles used as the solvents for bretylium and for tranylcypromine were diluted with saline, as in the case of the drug solutions, and infused for 5 min at 2 ml./min. These had no effect on the blood flow through the hand, on the reflex constrictor response to ice on the neck, nor on the magnitude of the hand vessel responses to tyramine, methylamphetamine or ephedrine. The latter responses were constrictions of 33·0%, 52·1% and 41·1% before tranylcypromine vehicle and 36·1%, 57·2% and 43·0%, respectively, after tranylcypromine. In the case of the vehicle for bretylium the corresponding values were: before 45·2%, 53·7% and 56·6% and after 39·2%, 55·4% and 54·4%.

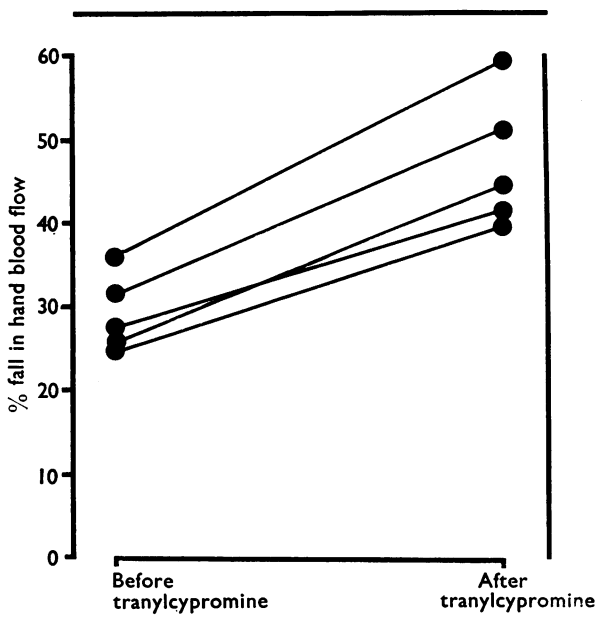

FIG. 4. Per cent fall in hand blood flow in each of five subjects in response to noradrenaline (50 ng min) before and after tranylcypromine (50 μg/min for 5 min).

Discussion

In the present study the striking feature is the marked enhancement of the constrictor effect of tyramine and methylamphetamine, and to a lesser extent of ephedrine, on the hand blood vessels in the presence of bretylium tosylate. The effect of bretylium alone on these vessels was to block reflexly induced sympathetic activity, vasoconstriction no longer being produced in the bretylium-treated hand when ice was applied to the subject's neck.

This finding is in keeping with that of Burn & Rand (1960) who observed that the action of tyramine in constricting the nictitating membrane of the spinal cat was more prolonged after administration of bretylium at a time when the response to sympathetic nerve stimulation was abolished. Huckovic (1960) found a similar effect with tyramine after bretylium in the perfused rabbit ear and the isolated atrium, but the response to amphetamine on the latter was not potentiated.

Wilson & Long (1960) demonstrated that when bretylium was administered to hypertensive patients on amphetamine therapy for weight reduction, no hypotensive response to the drug could be obtained. This effect was attributed to antagonism of the hypotensive action of bretylium by amphetamine. In view of the results of the present investigation, it might also be related to the potentiating action of bretylium on the peripheral vascular action of the amine.

The mechanism of enhancement of tyramine's constrictor response by bretylium is not clear. It is unlikely to be related to continuous release of noradrenaline from the nerve endings because at the time that the tyramine and other sympathomimetic amines were given the constrictor effect of bretylium had worn off and the flow had returned to, or slightly above, the resting level. At this time, also, it has been shown that there is no change in sensitivity of the vessels to infused noradrenaline (Cooper *et al.*, 1963).

The enhancement may be related to the monoamine oxidase inhibiting property which bretylium is said to possess. There is recent evidence to support such a facet of the action of bretylium (Giachetti & Shore, 1967). The effect of such an action would be twofold: (*a*) to protect tyramine from degradation by monoamine oxidases as it passes into the noradrenaline store and (*b*) to protect the noradrenaline that is released by tyramine from inactivation by the enzyme within the nerve ending.

These two effects, taken together, would mean that a greater amount of noradrenaline would be released from the storage sites by the sympathomimetic agent in the presence of bretylium. This conclusion has also been reached by Pettinger & Oates (1968) from the results of studies which showed that reduction of the metabolism of tyramine within the nerve ending is a major mechanism in the supersensitivity to this amine during monoamine oxidase inhibition.

While reduction in breakdown as a result of monoamine oxidase inhibition might account, at least in part, for the potentiation of tyramine's action, such a mechanism would be unlikely to apply in the case of the other sympathomimetics, methylamphetamine and ephedrine, which are not substrates for the enzyme. In the case of these drugs the potentiation must be accounted for in other ways, and it has been suggested that potentiation of their action by monoamine oxidase inhibitors may be due to inhibition of intraneuronal breakdown of transmitter providing an enhancement of the store available for release (Pettinger & Oates, 1968).

Rand & Trinker (1968) have presented evidence to show that monoamine oxidase

inhibitors potentiate the pressor responses of indirectly acting sympathomimetic amines, not by interfering with the metabolism of endogenous noradrenaline, but by retarding the binding or breakdown of these amines within the liver microsomal enzyme system. In the present experiments, however, bretylium and tranylcypromine potentiated the constrictor action of tyramine, methylamphetamine and ephedrine on the blood vessels of the hand when the drugs were given by local arterial injection, and this effect cannot be attributed to any action on the liver. If the potentiating effect of bretylium is due to monoamine oxidase inhibition, this must be a local action at the peripheral nerve endings or vessel wall.

It is not clear why the sympathetic nerves, after bretylium treatment, are capable of releasing transmitter in response to the indirectly acting sympathomimetic amines and yet do not do so in response to reflex activation. It may be that nerve impulses and the amines release transmitter either by different release mechanisms or from separate stores within the nerve ending. A selective blocking action of bretylium on the nervously activated store or mechanism, coupled with monoamine oxidase inhibiting action on the amine activated store or mechanism, could account for the observed effect.

The potentiation of the response of the hand vessels to the sympathomimetic amines by tranylcypromine could, as in the case of bretylium, be attributed to its monoamine oxidase inhibiting property. However, unlike bretylium, tranylcypromine also increases the sensitivity of the vessels to noradrenaline, and this could contribute to its potentiating effect on the action of the amines.

The parallelism in the pattern of enhancement of the constrictor response of the hand blood vessels to tyramine, methylamphetamine and ephedrine in the presence of both bretylium and tranylcypromine suggests that monoamine oxidase inhibition may be a common factor in their potentiating actions.

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