SOME ACTIONS OF METHYLENE BLUE AND OF MEPYRAMINE IN THE BODY

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Methylene blue is known to inhibit the action of amine oxidase *in vitro*, without affecting the action of other oxidases and dehydrogenases. The pressor effect of adrenaline injected into the splenic vein is much increased by the simultaneous injection of methylene blue, and Philpot and Cantoni[1] concluded that the amine oxidase in the liver is inhibited by such treatment. This conclusion is borne out by the observation that the potentiating effect of methylene blue on the pressor response to adrenaline is much weaker when given by the jugular route than when given directly into the portal system. Blaschko and Burn[2] have recently shown that amine oxidase from rabbit liver has a preference *in vitro* for noradrenaline as compared with adrenaline. It seemed of interest therefore to see if the pressor effect of noradrenaline injected into the splenic vein is potentiated to a greater extent than is adrenaline by injections of methylene blue. The work of West[3] had suggested that relatively more adrenaline than noradrenaline is inactivated *in vivo* in the liver of cats and rabbits, so there is discrepancy between *in vitro* and *in vivo* results.

Besides the action of these amines on the blood pressure, we have also studied their effects on the nictitating membrane of the cat. Bülbring and Burn[4] showed that noradrenaline has little effect on the normal membrane but denervation enhances its action, even more than that of adrenaline. They suggest that chronic denervation results in a decline in the amine oxidase activity so that the two amines become equi-active. By giving methylene blue it might be possible to inhibit the amine oxidase in the normal membrane and show that noradrenaline becomes as active as adrenaline in producing excitation.

Methods

Cats were used throughout this study. In most experiments, the cats were made spinal and prepared by the method of Burn, Hutcheon and Parker[5], blood pressure being recorded from the left carotid artery. The suprarenal vessels of both glands were ligated, and cannulae inserted into the femoral vein and one of the splenic veins. Contractions of the right nictitating membrane were recorded isotonically, in some cases, 7 to 10 days after denervation by removal of the superior cervical ganglion. In three experiments on cats under chloralose anæsthesia, a T-shaped cannula[6] was inserted into the right carotid artery, so that the amines could be passed directly to the membrane. Chlorazol fast pink (0·1 g./kg.) was used as the anticoagulant. Methylene blue was used as a 0·002M solution. Solutions of *l*-adrenaline and *l*-noradrenaline were prepared in 0·01N hydrochloric acid.

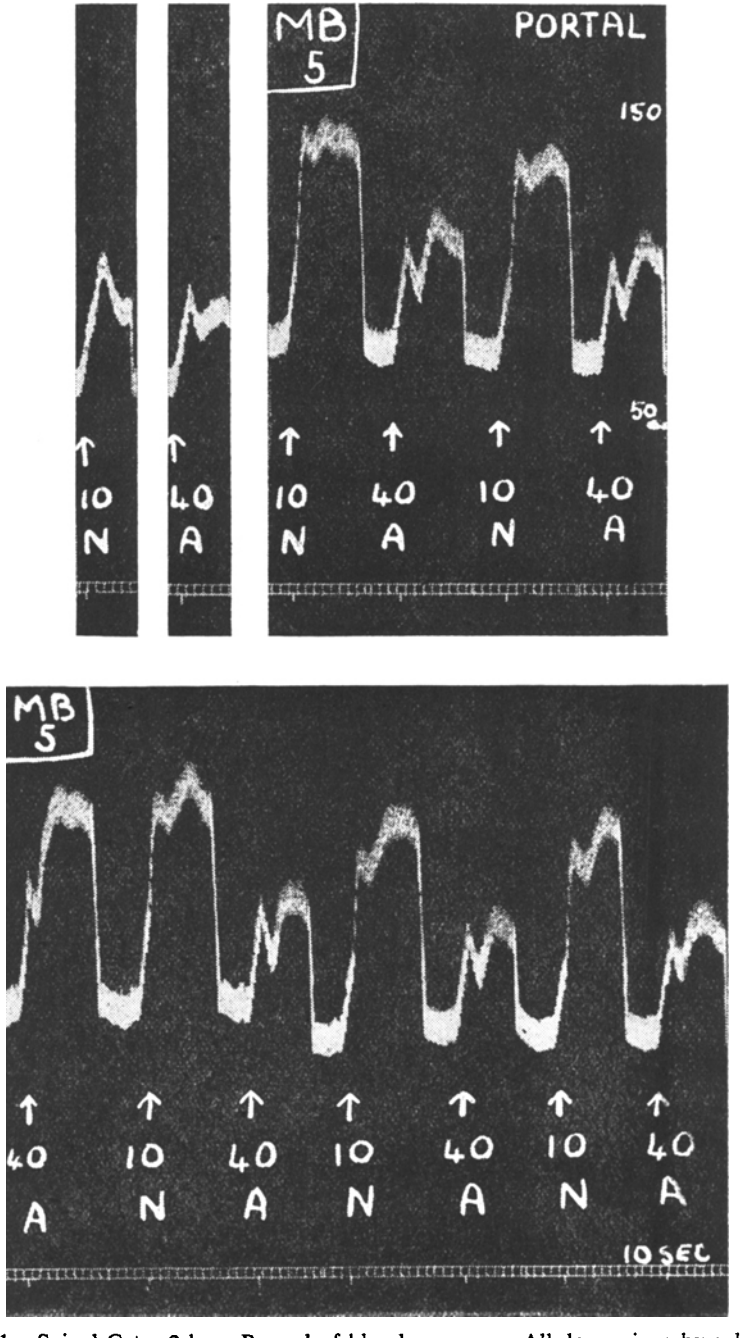

FIG. 1. Spinal Cat. 2 kg. Record of blood pressure. All doses given by splenic vein. Note that all doses of noradrenaline (N 10 μg.) are potentiated by methylene blue (MB), whereas only one dose of adrenaline (A 40 μg.) is potentiated (lower tracing). Time in 10 sec.

RESULTS

Intraportal doses of methylene blue. When a small dose of methylene blue (5 ml. of a 0·002M solution) is given by splenic vein, the actions of subsequent intraportal doses of noradrenaline are greatly potentiated for more than 1 hour, whereas only the first subsequent intraportal dose of adrenaline is increased. This effect is well shown in Figure 1. Adrenaline therefore may be potentiated if given immediately after methylene blue, but the potentiation is not maintained.

When larger doses of methylene blue are given by splenic vein, the actions of intraportal doses of both adrenaline and noradrenaline on both the blood pressure and the nictitating membrane are potentiated (Figure 2). In fact, equipressor doses of the amines by this route are

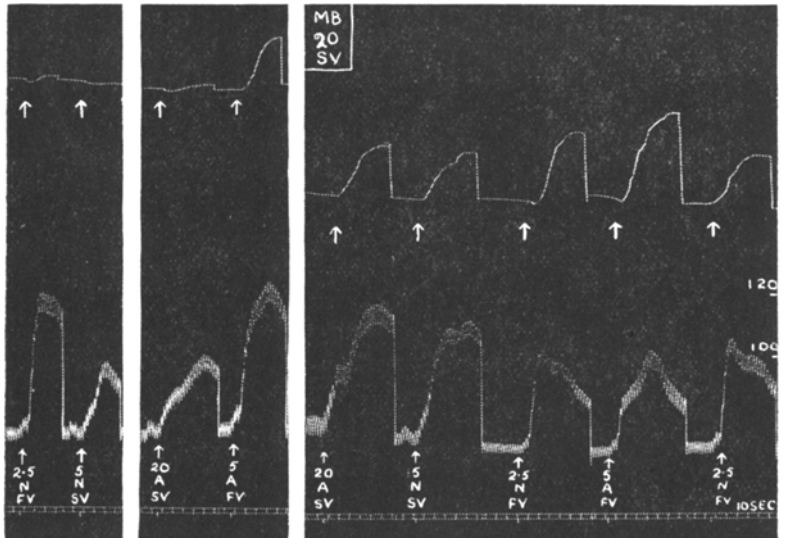

FIG. 2. Spinal Cat. 1·5 kg. Upper record, normal nictitating membrane; lower record, blood pressure. Note that, after an intraportal dose of methylene blue (MB), intraportal adrenaline (A 20 μg. S.V.) and noradrenaline (N 5 μg. S.V.) are now equally active on blood pressure and nictitating membrane. Similarly, intravenous adrenaline (A 5 μg. F.V.) and noradrenaline (N 2·5 μg. F.V.) are equally active after methylene blue. Time in 10 sec.

now equi-active on the membrane. Sinct noradrenaline is only about one-quarter as active as adrenaline in causing excitation of the normal membrane, this result suggests that methylene blue has inhibited amine oxidase in the membrane. The conclusion is strengthened by the results of intravenous doses (femoral vein) of the amines. The large intraportal dose of methylene blue has potentiated these actions of adrenaline and noradrenaline on the membrane so that they are now equi-active, whilst at the same time the actions on the blood pressure are both correspondingly reduced (Figure 2). We feel this is clear evidence that methylene blue can inhibit amine oxidase in the normal nictitating membrane and so greatly potentiate the action of noradrenaline.

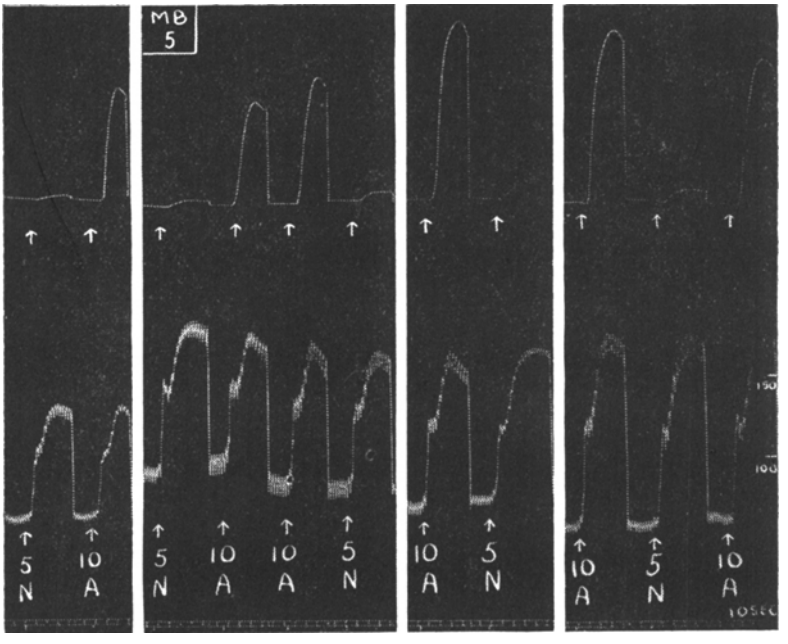

FIG. 3. Spinal Cat. 3·5 kg. Records as Fig. 2. All doses given by femoral vein. Note the slow potentiation of the adrenaline effect (10 μg. A) and noradrenaline effect (5 μg. N) on the membrane.

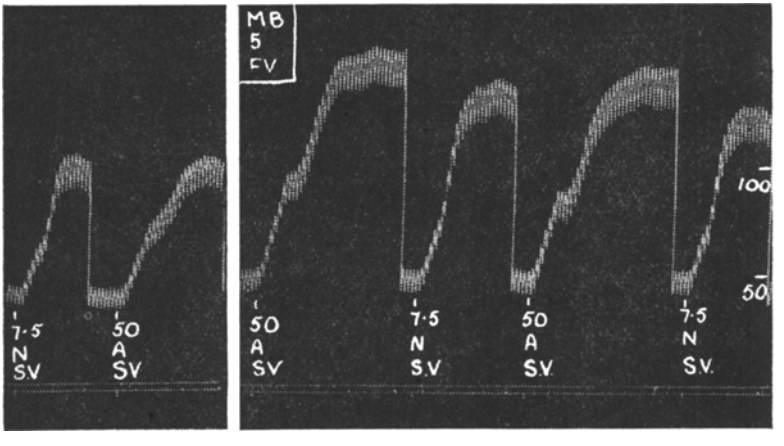

FIG. 4. Spinal Cat. 2·3 kg. Blood pressure record. Note that intravenous methylene blue (MB) potentiates equally intraportal adrenaline (50 μg. A) and noradrenaline (7·5 μg. N). Time in 10 sec.

Intravenous doses of methylene blue. When a small dose of methylene blue (5 ml. of a 0·002M solution) is given by femoral vein, there is potentiation of the actions of intravenous doses of both adrenaline and noradrenaline on the blood pressure and nictitating membrane (Fig. 3).

The effect on the membrane is slow to reach its maximum, and may in certain circumstances be preceded by a depression of the normal responses. A similar intravenous dose of methylene blue potentiates the pressor action of intraportal doses of both amines to the same extent (Figure 4).

In three cats under chloralose anæsthesia, noradrenaline was found to be as active as adrenaline on the chronically denervated nictitating membrane. Intraportal or intra-arterial doses of methylene blue had little effect on these actions as was expected if chronic denervation has led to a decline in the amine oxidase content.

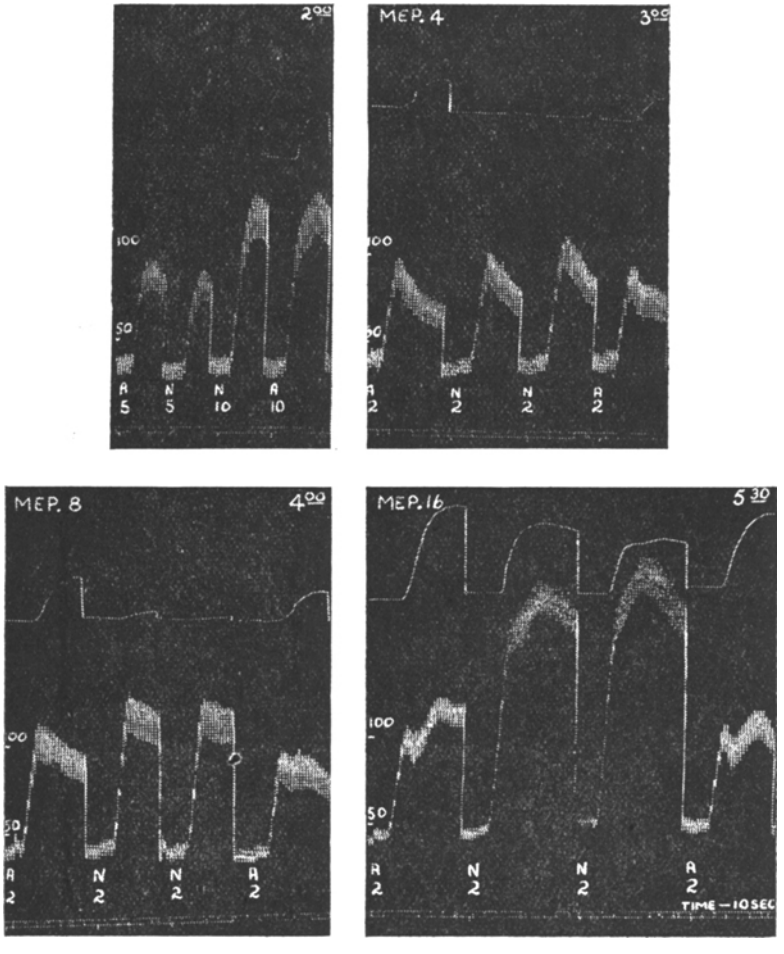

FIG. 5. Spinal Cat. 2 kg. Records as Fig 2. All doses given by femoral vein. Small doses of mepyramine (4 and 8 mg./kg.) potentiate equally the effects of adrenaline (2 μg. A.) and noradrenaline (2 μg. N). A further large dose (16 mg./kg.) potentiates noradrenaline much more than adrenaline.

Effect of mepyramine on adrenaline and noradrenaline. In 3 further spinal cats, a result suggestive of amine oxidase inhibition has been observed using mepyramine. Small intravenous doses up to 4 mg./kg. potentiate the actions of adrenaline and noradrenaline on the blood pressure, in a manner similar to that previously reported[7,8]. But when larger doses of mepyramine are administered (up to 28 mg./kg.) the action of noradrenaline is greatly potentiated both on blood pressure and nictitating membrane whilst that of adrenaline is increased to a much smaller extent (Figure 5). Mepyramine therefore makes the cat much more sensitive to noradrenaline than to adrenaline.

Discussion

Adrenaline and noradrenaline are present in extracts of animal and human tissues and are liberated in the body when adrenergic nerves are stimulated. One of the processes of inactivation of these substances in the body is oxidation by amine oxidase, an enzyme present in many tissues, including the liver and nictitating membrane. Earlier workers had shown that methylene blue can inhibit the amine oxidase in the liver, and the results of the experiments reported here suggest that it can also inhibit the enzyme in the normal nictitating membrane.

Since noradrenaline is the natural adrenergic mediator in the liver[9], it is to be expected that an efficient enzyme system is present there to inactivate it. The failure of noradrenaline, when given by splenic vein, to be inactivated by the liver as quickly as adrenaline may be due either to amine oxidase not being the chief source of inactivation of noradrenaline or to slow absorption from the blood stream during its passage through the liver sinusoids[3]. These suggestions may help to explain why intraportal doses of noradrenaline are greatly potentiated by methylene blue, whereas corresponding doses of adrenaline are only slightly increased, but the effects appear to be very complex, particularly on the blood pressure.

On the nictitating membrane, on the other hand, it is clear that intraportal doses of methylene blue potentiate the action of noradrenaline more than that of adrenaline. Since now equi-pressor doses of the two amines are equally active on the membrane, the amine oxidase in the membrane must be inhibited. The observed effect is similar to that seen after chronic denervation when the amine oxidase content has decreased considerably.

Small doses of the antihistamine mepyramine potentiate the actions of adrenaline and noradrenaline on the blood pressure and nictitating membrane of spinal cats. Larger doses, however, usually reduce the effects of adrenaline and noradrenaline[8]. There is no simple explanation why very large doses of mepyramine potentiate noradrenaline more than adrenaline. Inhibition of amine oxidase may be a substantial part of the effect, but, as with the methylene blue observation, there are probably other factors influencing the results.

SUMMARY

1. Intraportal doses of methylene blue potentiate the pressor effects of adrenaline and noradrenaline in spinal cats. The action of noradrenaline on the normal nictitating membrane is potentiated more than that of adrenaline; methylene blue may therefore inhibit the action of amine oxidase in the nictitating membrane of cats. Corresponding intravenous doses of methylene blue do not appear to possess this action.

2. The actions of noradrenaline on the blood pressure and nictitating membrane of the cat is potentiated by large doses of mepyramine to a much greater extent than are those of adrenaline.

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