Brit. J. Pharmacol. (1952), 7, 338.

THE PHARMACOLOGICAL ACTIONS OF 6-METHYLADRENALINE

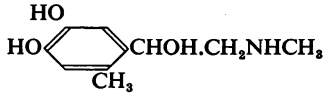
BY

R. SINGH GREWAL

From the Department of Pharmacology, University of Oxford

(Received February 9, 1952)

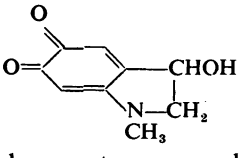
N-methyl-3 : 4-dihydroxy-6-methylphenylethanolamine or 6-methyladrenaline (I) was recently prepared by Dr. E. J. Forbes, under Sir Robert Robinson's direction, and I was given the opportunity of studying its pharmacological properties. The



(I)

main interest of this compound arises from its inability to undergo one of the chemical reactions postulated as responsible for the inactivation of adrenaline *in vivo*.

Adrenaline may be inactivated in the body in several ways : by the oxidative action of amine oxidase ; by the formation of adrenochrome (II) ; by the oxidative action of polyphenol oxidase ; and by conjugation as a catechol acid sulphate or glycuronide.



(II)

The last method is known to occur when adrenaline is administered orally (Richter, 1940 ; Beyer and Shapiro, 1945), but it may be only a subsidiary path of inactivation for physiologically liberated adrenaline. Inactivation by polyphenol oxidase has not yet been demonstrated *in vivo*.

The function of amine oxidase as an inactivating mechanism has been questioned by Bacq (1949), who thinks that adrenochrome formation is the main route by which adrenaline is inactivated. 6-Methyladrenaline, owing to the methyl group, is incapable of forming an adrenochrome-like compound, and Dr. H. Blaschko, who compared the oxidation of 6-methyladrenaline with that of adrenaline, found that potassium iodate did not oxidize it to an iodochrome type of compound. Consequently 6-methyladrenaline, simply because it is incapable of forming an adrenochrome-like compound, is a substance of considerable pharmacological interest.

Experimental Observations

Preparation of solution.—When dissolved in water in the ordinary way, 6-methyladrenaline (base) rapidly changed in colour, and steps were therefore taken to prepare

a stable solution before each experiment. Saline was first boiled to expel dissolved air, and was covered with liquid paraffin. A weighed portion of 6-methyladrenaline was dissolved on a watch glass in 0.1 N-HCl, and then made up to the required volume in a cylinder with the boiled saline. The solution was at once covered with liquid paraffin. Dilutions of this solution were also made with boiled saline. In many experiments, e.g. on the isolated heart, each injection was freshly prepared.

Action on the blood pressure.—When examined on the spinal cat the pressor effect of methyladrenaline resembled that of adrenaline or of noradrenaline, though when the doses produced the same rise of blood pressure the effect of methyladrenaline was more prolonged than that of either. This difference is shown in Fig. 1, which also shows that methyladrenaline caused a very prolonged contraction of the nictitating membrane. In eight experiments on the spinal cat, the dose of methyladrenaline causing the same rise of blood pressure as adrenaline varied from 10 to 50 times the dose of adrenaline, the mean figure being 21.

In the cat anaesthetized with chloralose, 6-methyladrenaline appeared to be more potent than in the spinal cat, the equipressor dose in four experiments ranging from 5 to 16 times the dose of adrenaline, the mean figure being 9.

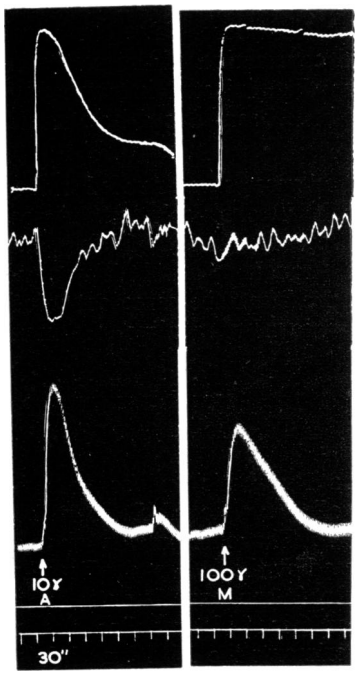


FIG. 1

FIG. 1.—Spinal cat. Top record, normal nictitating membrane; middle record, uterus; bottom record, arterial blood pressure. Injections of 10 μg. adrenaline (A), and of 100 μg. 6-methyladrenaline (M). Note that methyladrenaline caused a more prolonged contraction of the nictitating membrane than adrenaline, but had no action on the uterus.

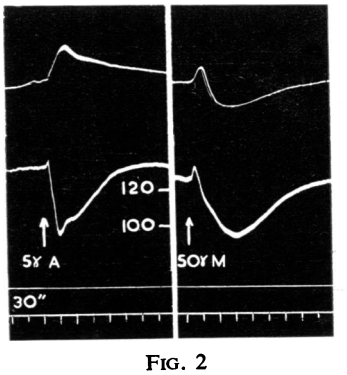


FIG. 2

FIG. 2.—Cat under ether, vagi cut. Upper record, volume of left hindleg, in which the sciatic nerve had been divided 8 days previously. Lower record, arterial blood pressure. Injections of 5 μg. adrenaline (A), and 50 μg. 6-methyladrenaline (M). Note that both injections caused a fall in blood pressure, but, whereas the hindleg was dilated when adrenaline was injected, it was constricted when 6-methyladrenaline was injected.

In occasional experiments it was observed that 6-methyladrenaline produced, after an initial rise, a fall of blood pressure as its main effect. With a larger dose the ordinary rise reappeared, but was followed by a fall.

In cats anaesthetized with ether, when the vagi were cut, this fall was commonly seen and resembled the fall produced by the injection of small amounts of adrenaline. In four cats plethysmograph records of the left hindleg were obtained, the sciatic nerve having been cut 7–9 days before in an aseptic operation. These cats were also examined under ether with the vagi cut, and Fig. 2 illustrates that 6-methyladrenaline did not cause dilatation of the hindleg as did adrenaline. Both substances lowered the blood pressure. Adrenaline causes the fall because of the dilatation of the muscle vessels; 6-methyladrenaline does not cause a fall in this way, and the decline in limb volume might appear to be passive. This, however, is not so, because in some experiments the same fall of limb volume accompanied a rise in blood pressure. The action of 6-methyladrenaline on the hindleg vessels was therefore constrictor.

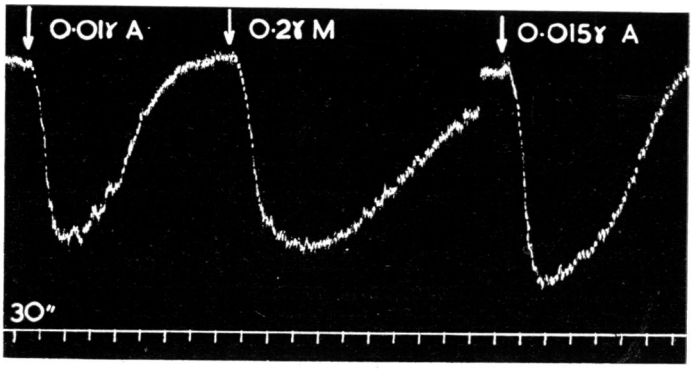
Another possible cause of the fall of blood pressure produced by 6-methyladrenaline was a depression of ganglionic transmission of vasoconstrictor impulses to the blood vessels. Adrenaline is known to have such an action. Two experiments were therefore performed in which the cervical sympathetic chain was divided and laid upon electrodes in a cat anaesthetized with chloralose. The chain was stimulated so as to produce a large and maintained contraction of the nictitating membrane. 6-Methyladrenaline (0.05–0.5 mg.) was then injected intravenously, but it had no effect on the contraction such as would have been caused by a small dose of hexamethonium.

Action on blood vessels.—The action on blood vessels was studied in the isolated rabbit ear perfused through the central artery with Locke's solution. A record was made of changes in outflow using Stephenson's recorder (1948). 6-Methyladrenaline had a similar constrictor action to adrenaline, though as a rule it was more prolonged, as shown in Fig. 3*a*. The dose which produced the same constriction was, in ten experiments, from 10 to 20 times the dose of adrenaline, the mean figure being 12.

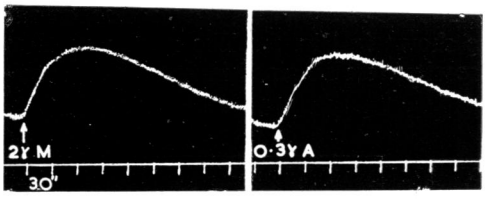
When benzylimidazoline (Priscol) was added to the perfusing fluid in a concentration 2×10^{-4}, the action of 6-methyladrenaline, like that of adrenaline, was reversed, as shown in Fig. 3*b*.

Action on the heart.—The action of sympathomimetic amines on the heart is known to vary; thus ephedrine has rarely any effect on the rabbit heart, but on the cat heart its stimulant action is regularly seen. The experiments were therefore carried out on the isolated heart of the cat perfused by the Langendorff method. The action was always weak and very variable. In fifteen experiments, the rate was sometimes increased with the first injection of 6-methyladrenaline and then unaffected by subsequent injections. In some experiments the injection of 6 μg. caused an increase, while the injection of larger amounts, 10 or 20 μg., had no effect; subsequently the smaller amount (6 μg.) again increased the rate. In other experiments the rate was unaffected by all amounts injected from 2–20 μg.

The variation is best illustrated by the fact that, when 4 μg. 6-methyladrenaline was injected on twelve occasions in twelve different preparations, it failed to modify the rate in six trials, while it increased the rate by 11, 14, and 33 per cent respec-



(a)

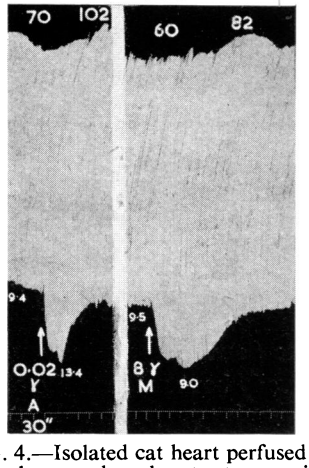


(b)

FIG. 3.—Record of outflow from vessels of rabbit ear perfused with Locke's solution. (*a*) 0.2 μg. 6-methyladrenaline (M) caused a constriction similar in size (but longer in duration) to that caused by 0.01 μg. adrenaline (A). (*b*) When the perfusion fluid contained benzylimidazoline (0.2×10^{-3}), the effect of both substances was reversed. 2 μg. methyladrenaline had a similar effect to that of 0.3 μg. adrenaline.

tively in three trials; in the remaining three trials there was a slight increase in the rate (2, 2, and 6 per cent). When 10 μg. 6-methyladrenaline was injected, it failed to affect the rate in six out of seven trials. When an increase of rate was obtained and compared with that produced by adrenaline, the result, illustrated in Fig. 4*a*, showed that 6-methyladrenaline was from 200 to 1,200 times weaker than adrenaline, though in one experiment a figure of 30 times was obtained.

(a)



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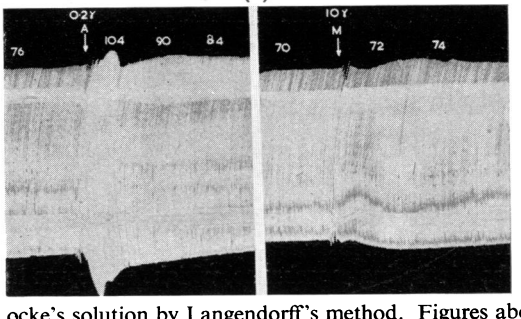

FIG. 4.—Isolated cat heart perfused with Locke's solution by Langendorff's method. Figures above the record are heart rate per min. Figures below are outflow in ml./min. (*a*) In this preparation the injection of 8 μg. 6-methyladrenaline (M) caused an increase in amplitude and rate similar to that caused by 0.02 μg. adrenaline (A). (*b*) In this preparation, 10 μg. 6-methyladrenaline (M) had a very slight effect on the amplitude and rate.

The effect on the amplitude of contraction was more regular than the effect on the rate, and it was more prolonged. The result shown in Fig. 4*b* was, however, seen as often as that in Fig. 4*a*. The mean increases produced by the amounts 2, 4, and 6 μg. were 9, 11, and 30 per cent respectively, but larger doses, 10 and 20 μg., had less effect, increasing the amplitude by 17 and 16 per cent respectively. Even so, in relation to the effect of adrenaline, the effect of 6-methyladrenaline on the amplitude was very weak, and in no way corresponded to the relative vasoconstrictor action.

Action on isolated auricles.—When added to a bath containing the isolated auricles of the rabbit, 6-methyladrenaline in concentrations up to 0.2×10^{-5} had no action. On auricles stimulated electrically by the method described by Dawes (1946), 6-methyladrenaline acted differently from adrenaline in that it failed to increase the maximum rate at which the auricles would follow an applied stimulus. The action of adrenaline has been described by Elío (1947). 6-Methyladrenaline, on the contrary, depressed the maximum rate, as shown in Fig. 5. The results of three experiments are given in Table I ; similar results were obtained in five other experiments.

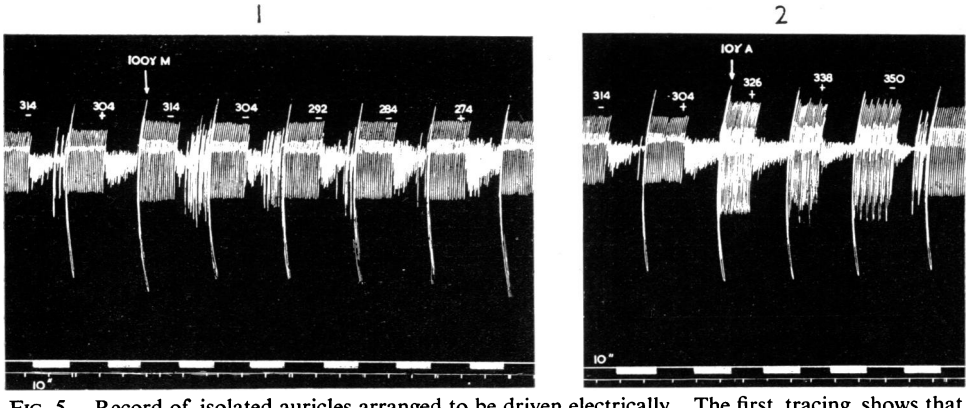

FIG. 5.—Record of isolated auricles arranged to be driven electrically. The first tracing shows that 100 μg. 6-methyladrenaline reduced the maximum rate at which the auricles would follow an applied stimulus from 304 to 274 per min. The second tracing shows that 10 μg. adrenaline increased the maximum rate from 304 to 338 per min.

Action on coronary vessels.—The effect of 6-methyladrenaline on the outflow in the experiments made with the Langendorff preparation showed that in four out of seven experiments it increased the coronary flow as did adrenaline. In the other three experiments 6-methyladrenaline decreased the coronary flow ; in one of these adrenaline also decreased the coronary flow (compare Kordik, 1951), in one adrenaline had no effect, and in one adrenaline increased the flow.

Nictitating membrane and pupil.—Reference has already been made to the effect on the nictitating membrane, which, as shown in Fig. 1, is very prolonged. In seven experiments the mean figure for the amount of 6-methyladrenaline producing the same height of contraction as a given amount of adrenaline was seven times as great. In four cats the superior cervical ganglion was removed on the right side, and 7–9 days later the contractions of the normal and denervated membranes were recorded

TABLE I

EFFECT OF ADRENALINE AND 6-METHYLADRENALINE ON MAXIMUM RATE OF DRIVEN AURICLES OF THE RABBIT

Bath 100 ml.

Exp.	Adrenaline		6-Methyladrenaline	
	Dose μg.	Effect (%)	Dose μg.	Effect (%)
1	10	+17.3	100	−15.3
			200	−12.1
2	10	+21	100	−8.9
			200	−8.2
			400	−12.6
3	10	+9.8	100	−17.1
			200	−28.6

in the spinal preparation. Fig. 6 shows side by side the effects of injecting adrenaline and 6-methyladrenaline. The denervated membrane contracted more in response to 6-methyladrenaline than did the normal membrane, but the difference was much less than the difference for adrenaline. In both membranes the effect of 6-methyladrenaline was more prolonged than that of adrenaline.

On the pupil the action of 6-methyladrenaline was weak ; in two cats in which eight and seven observations were respectively made, the amounts of 6-methyladrenaline having the same effect by intravenous injection as a given amount of adrenaline were 60 and 62.5 times as great.

Intestinal balloon.—A balloon was prepared by attaching a rubber fingerstall to a catheter and filling it with water ; the balloon was then inserted into the proximal end of the small intestine of a cat under chloralose anaesthesia. Injections of 6-methyladrenaline and of adrenaline were given when the pressure changes in the balloon shown by a piston recorder were sufficiently regular. Seven experiments were performed, and in the last experiment both adrenal glands were excluded from the circulation.

In causing inhibition of intestinal movement, 6-methyladrenaline was more powerful, relative to adrenaline, than in any other action. Fig. 7 shows that 10 μg. 6-methyladrenaline caused a more prolonged inhibition than either 2 μg. or 4 μg. adrenaline. The mean figure from the seven experiments for the amount of 6-methyladrenaline producing the same inhibition as a given amount of adrenaline was 2.55 times as great.

Peristalsis in guinea-pig ileum.—Observations were also made on the Trendelenburg preparation of the guinea-pig ileum *in vitro*, in which the peristaltic waves induced by raising the pressure in the lumen of the gut were recorded. Doses of adrenaline and of 6-methyladrenaline just sufficient to inhibit peristalsis were determined. In four experiments the mean figure for 6-methyladrenaline was 6.5 times as great as for adrenaline.

Pendular movements.—On the isolated duodenum of the cat and of the rabbit the inhibitory action of methyladrenaline was very much weaker. The mean figure for an amount of 6-methyladrenaline having an equivalent action to an amount of adrenaline was about 200 times greater both for cat and for rabbit duodenum.

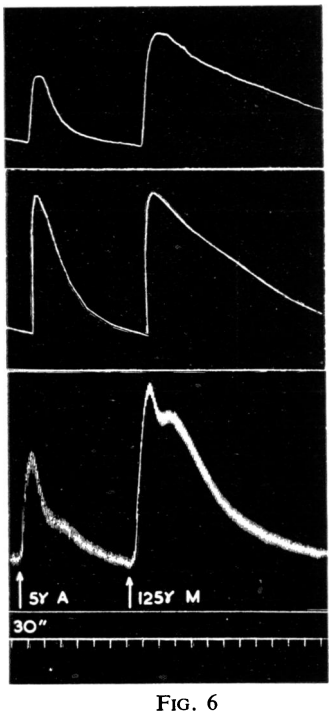

FIG. 6

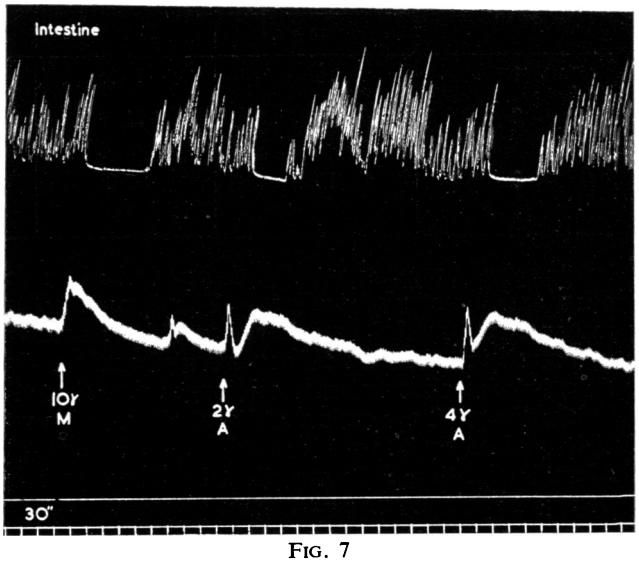

FIG. 7

FIG. 6.—Spinal cat. Comparison of the action of 5 μg. adrenaline (A) and of 125 μg. 6-methyladrenaline (M) on the normal nictitating membrane (top tracing), on the denervated nictitating membrane (middle tracing), and on the blood pressure (bottom tracing). Both substances had about the same effect on the denervated membrane, but with each substance this was greater than the effect on the normally innervated membrane.

FIG. 7.—Cat under chloralose. Upper tracing, record of pressure changes in intestinal balloon inserted in the duodenum. Lower tracing, arterial blood pressure. Note that 10 μg. 6-methyladrenaline (M) caused an inhibition greater than that caused by 2 μg. adrenaline (A) or than that caused by 4 μg. adrenaline.

Blood sugar.—Experiments were carried out to compare the action of adrenaline and of 6-methyladrenaline on the blood sugar. After preliminary observations were made, it was decided to test the effect of 60 μg. adrenaline and of 1 mg. 6-methyladrenaline. Each substance was injected subcutaneously in the dose stated into each of two rabbits, as shown in Table II. The rabbits, which weighed 2.4 and 2.1

TABLE II

RISE OF BLOOD SUGAR IN THE RABBIT PRODUCED BY ADRENALINE AND 6-METHYLADRENALINE

Drug and dose	Percentage hyperglycaemia in Rabbit 1	Rabbit 2
0.06 mg. adrenaline ..	70, 25.6, 57.7 (Mean, 51.1)	77.5, 65 (Mean, 71.2)
1 mg. 6-methyladrenaline ..	45.5, 57, 57 (Mean, 53)	47, 77 (Mean, 62)

kg. respectively, were kept without food overnight. A sample of blood was taken before the injection was given, and again at hourly intervals for 5 hr. The blood sugar was determined by precipitating the proteins according to Somogyi's method, and estimating the sugar by the method of Hagedorn-Jensen. The mean blood sugar values for the five samples after injection were calculated, and the rises of blood sugar were expressed as percentages of the initial values. The percentage hyperglycaemia in the different experiments is shown in Table II. The mean figure for the percentage hyperglycaemia caused by 0.06 mg. adrenaline in the two rabbits is 61 as compared with 58 for 1 mg. 6-methyladrenaline. Thus 6-methyladrenaline has a similar action in 16 times the dose.

Rabbit uterus.—The action of adrenaline on the isolated rabbit uterus is motor, and 6-methyladrenaline was found to have a similar motor effect. A comparison with adrenaline was made on one horn of the uterus of five rabbits, and it was found that the mean figure for the dose exerting a given effect was 23 times as great as the dose of adrenaline. The five figures varied from 3.5 to 33.3, though four were between 20 and 33. The effect of 6-methyladrenaline was abolished like that of adrenaline in the presence of 10^{-6} ergotamine tartrate.

Spleen.—Observations were made on the spleen with its natural circulation by withdrawing it through an incision in the side of the cat and enclosing it in a plethysmograph ; the volume changes were recorded by a piston recorder. One cat was anaesthetized with chloralose ; the rest were spinal preparations. In the cat under chloralose and in one spinal cat, the doses of 6-methyladrenaline causing the same contraction as a dose of adrenaline were respectively 5 and 10 times as great. In the other three spinal cats, however, the doses were 50, 100, and 200 times as great. The effect of 6-methyladrenaline was of greater duration than that of adrenaline. Thus the mean figure of the different experiments was 73, though the very wide variation detracts from the value of this mean.

Cat uterus.—The cat uterus was used *in situ* in four experiments, and isolated in a bath in two experiments. In none of the experiments was 6-methyladrenaline observed to cause inhibition, though in each the effect of small amounts of adrenaline was inhibitory.

Bronchioles.—The action on the bronchioles was studied using the Konzett-Roessler apparatus in the guinea-pig anaesthetized with urethane. The inhibitory effect was very weak here also. Acetylcholine was injected in a dose of 8–10 μg.; this caused a transient bronchoconstriction. When adrenaline or 6-methyladrenaline was injected 45 sec. before the acetylcholine, the bronchoconstriction was less. In three experiments the amounts of 6-methyladrenaline required to produce the same effect as adrenaline were respectively 50, 80, and 200 times as great.

Skeletal muscle.—Experiments were carried out on the sciatic-gastrocnemius preparation of the cat anaesthetized with chloralose. The method used was that described by Bülbring and Burn (1942), in which the detached tendo Achillis is arranged to pull on a tension lever, the sciatic nerve being stimulated at regular intervals by maximal single shocks. When contractions of regular height were obtained, an injection of neostigmine (usually 10 μg.) was made into the central cut end of the external iliac artery of the opposite side, the injection being made almost into the bifurcation of the aorta so that the fluid was carried to the stimulated

muscle with little dilution. The injection caused a rise of tension as shown in Fig. 8, and, when this rise had reached its peak and was declining, an injection of 6-methyladrenaline or of adrenaline was made. Adrenaline in amounts of 10–20 μg. usually increased the tension again. 6-Methyladrenaline was without effect in amounts from 0.1 to 0.5 mg., but, in two experiments out of five, 1 mg. 6-methyladrenaline had a small effect, as shown in Fig. 8. Thus 6-methyladrenaline had very little action compared with adrenaline.

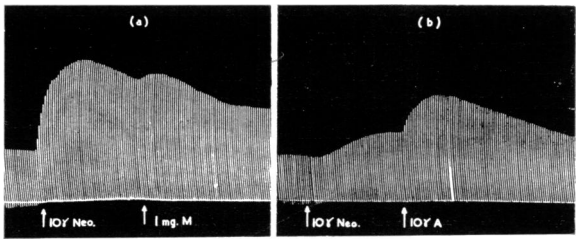

FIG. 8.—Record of twitch tension in gastrocnemius muscle of cat under chloralose caused by single shocks applied to the sciatic nerve. In (*a*) the injection of 10 μg. neostigmine (Neo) given into the bifurcation of the aorta caused an increase in the tension, and, as this declined, the injection of 1 mg. 6-methyladrenaline (M) by the same route caused a slight further increase. In (*b*) the augmentation due to 10 μg. adrenaline (A), again injected after 10 μg. neostigmine, is shown.

Bülbring preparation.—Experiments were also carried out on the Bülbring preparation of the phrenic nerve-diaphragm of the rat. In this also the effect of adrenaline was tested after the addition of neostigmine in a concentration 2.5×10^{-9}, which produced a steady increase in the contractions. A concentration of 4×10^{-7} adrenaline, applied after washing out the neostigmine, caused a prompt increase in the contractions. When 6-methyladrenaline was tested, no augmentor effect was recorded even with doses of 2 mg.

DISCUSSION

The action of 6-methyladrenaline differs from that of adrenaline chiefly in affecting the isolated heart so little. In addition it lacks an inhibitory action on the non-pregnant uterus of the cat, and is weak as a bronchodilator ; on the other hand, it has a fairly powerful inhibitory action on the intestine. Since it possesses most of the other actions of adrenaline, it might be concluded that its effect is exerted primarily on the receptors which Ahlquist (1948) has called "α" receptors, and that it has little or no action on "β" receptors. The action on blood vessels, and in various other tissues where it exerts a motor or stimulant action, is frequently of the order of 1/12th that of *l*-adrenaline. Blaschko (1950) has pointed out that both *d*-adrenaline and epinine have about 1/12th of the action of *l*-adrenaline also, and that this results from the less firm combination with the receptors they are able to effect. He suggests that *l*-adrenaline has three points of anchorage, whereas *d*-adrenaline and epinine have only two. A similar explanation may hold for 6-methyladrenaline.

The suggestion that 6-methyladrenaline acts only on "α" receptors and not on "β" receptors is, however, not supported by the fact that 6-methyladrenaline

possesses vasodilator effects ; these are seen in the isolated vessels of the rabbit ear, when perfused with Lock's solution containing benzylimidazoline, and as a fall in blood pressure when 6-methyladrenaline is injected into a cat, anaesthetized with ether, in which the vagi have been cut.

Since 6-methyladrenaline cannot form an adrenochrome-like compound, its metabolic fate must lie in a different direction. An attempt to discover whether it was a substrate of amine oxidase was unsuccessful, since the uptake of oxygen in the absence of amine oxidase was too great. However, the prolonged action it exerts on the nictitating membrane, whether denervated or not, suggests that it is removed from its site of action by diffusion rather than by enzymic destruction, though when the dose is so large this may be a wrong conclusion. The contrast between 6-methyladrenaline and adrenaline is well shown in Fig. 6. This figure shows, moreover, that 6-methyladrenaline causes a rather greater contraction of the denervated membrane than of the normal membrane, though the difference is very much less than with adrenaline. The size of the difference may again depend on the dose.

While 6-methyladrenaline is unstable *in vitro,* it may be less so in the body, and it might be an interesting compound from the point of view of therapeutics, since it has so little action on the heart. Many adrenaline derivatives have a limited usefulness because of the increase in the rate and force of the heart beat which they produce. A derivative which had little or no cardiac action would therefore be very useful. Unfortunately, 6-methyladrenaline has a very weak dilator action on the bronchioles. If this action could be intensified by altering the substituent attached to the N-atom, and if the compound could be rendered more stable, a useful therapeutic agent might result.

Summary

1. The pharmacological properties of N-methyl-3 : 4-dihydroxy-6-methylphenyl-ethanolamine (6-methyladrenaline) have been examined. The compound is of interest because it cannot form an adrenochrome-like compound and the blocking of this pathway of degradation may modify its properties.

2. 6-Methyladrenaline can be said briefly to possess the stimulant actions of adrenaline, with the exception of that on the heart, and to possess also the inhibitory action on the intestine. It lacks the inhibitory action on the uterus of the cat, which is so characteristic of adrenaline, and it is much weaker than adrenaline as a bronchodilator.

3. The structures on which the action of 6-methyladrenaline has been examined can be divided into three groups.

On the blood pressure, on isolated blood vessels, on the nictitating membrane, in producing hyperglycaemia, on the rabbit uterus, and on the intestine, the action of 6-methyladrenaline is similar to that of adrenaline, but is from 2.5 to 23 times weaker according to the organ.

On the cat uterus it has no action, and on the heart a very weak and inconstant action.

On the spleen, on the iris of the cat, and on the bronchioles, the action is much weaker than in the first group.

I am deeply indebted to Professor J. H. Burn for his guidance and encouragement at all the stages of this work.

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