Brit. J. Pharmacol. (1961), **17**, 124–130.

PERIPHERAL ACTIONS OF HEXAMETHONIUM IN RELATION TO THE DECREASING EFFECTS OF REPEATED DOSES ON THE BLOOD PRESSURE OF ANAESTHETIZED RATS

BY

J. G. BLACKMAN* AND R. LAVERTY

From the Department of Medicine, Otago University, Dunedin, New Zealand

(*Received June* 2, 1961)

When hexamethonium was given intravenously to anaesthetized rats, the depressor response produced by successive doses became less. This effect was not related to the duration of anaesthesia and the possible recovery of cardiovascular reflex activity. When the rat's own blood was perfused at constant rate through one hind limb, successive doses of hexamethonium reduced the hind-limb perfusion pressure to the same extent each time, but their effect on the blood pressure of the remainder of the animal became less. When the hind limb was perfused at constant rate with blood from a separate donor rat, hexamethonium in the donor blood produced a very small increase in the vasoconstrictor response to noradrenaline in the innervated hind limb but not in the denervated hind limb.

Evidence that hexamethonium and pentolinium can sensitize the blood vessels of the hind limb of the cat to the constrictor effects of adrenaline and noradrenaline has been given by Zaimis (1955, 1956) and by Mantegazza, Tyler & Zaimis (1958). They have suggested that this sensitizing action might explain (1) why hexamethonium brings about potentiation of the pressor effects of adrenaline and noradrenaline in the whole animal, (2) why the blood pressure response to repeated doses of hexamethonium becomes less in anaesthetized animals, and (3) why "tolerance" develops to the blood pressure lowering action of many ganglion-blocking compounds in man.

In the following experiments, the modification of the depressor response to successive doses of hexamethonium has been studied in the anaesthetized rat, and concurrent changes in the peripheral resistance of one of the hind limbs have been measured by a technique based on constant-rate blood-perfusion. The peripheral effect of hexamethonium on the sensitivity of blood-perfused denervated and innervated hind-limb blood vessels to noradrenaline has also been studied.

METHODS

Adult male albino rats (280 to 380 g) were used throughout. Arterial blood pressure was recorded by a small-volume mercury manometer connected to the femoral artery by a polythene tube filled with heparin-saline (250 u./ml.). In most experiments rats were anaesthetized with chloralose (50 to 60 mg/kg) injected intravenously. In one group, more prolonged

* Present address: Department of Pharmacology, University of Edinburgh.

anaesthesia was produced by injecting urethane (600 to 700 mg/kg) subcutaneously 15 min after the chloralose. The rats were kept warm by overhead electric lamps.

Hind-limb perfusion experiments

Perfusion with animal's own blood. In these experiments the hind limb was perfused through the femoral artery at constant rate with blood taken from the opposite femoral artery. The hind limb was either left intact—this ensured normal nervous vasomotor tone in the hind limb and minimal blood loss and surgical trauma—or, alternatively, the hind limb was isolated at a level just proximal to the third trochanter of the femur, except for the femoral vein connexion, which was retained. The femoral and sciatic nerves were kept intact, every care being taken to maintain as complete hind-limb nervous tone and haemostasis of the cut surfaces as possible. With these preparations, the effect of hexamethonium on the hind-limb "peripheral resistance," as indicated by the perfusion pressure, could be studied independently of the blood pressure of the remainder of the animal.

Perfusion with blood from donor. In these experiments, the hind limb with its vascular system isolated was perfused at constant rate with blood from a separate donor rat (Field & Laverty, 1958). In some experiments, the femoral and sciatic nerves were kept intact, so that central vasomotor control was retained; in other experiments, the hind limb was completely isolated by cutting the nerves. With these preparations, the peripheral effect of hexamethonium on the vascular response of the hind limb to noradrenaline could be measured.

The response to noradrenaline was measured as the rise in perfusion pressure induced by injecting noradrenaline hydrogen tartrate (equivalent to 0.01 μg base in 0.05 ml. of 0.9% sodium chloride solution) into the perfused hind limb. Records were obtained of at least two responses to noradrenaline given at 10 min intervals at the inflow to the perfused limb. Hexamethonium bromide was injected into the blood perfusing the hind limb or intravenously into the donor animal. In either case, the dose was large (3 to 5 mg) and corresponded to an initial concentration in the perfusing blood of approximately 100 to 150 μg/ml. In most experiments, hexamethonium was given twice. Hind-limb perfusion pressures were recorded immediately before each noradrenaline injection.

In all perfusion experiments, heparin was used as anticoagulant. As with the blood pressure, the hind-limb perfusion pressure was recorded with a small-volume mercury manometer. The constant-rate blood-perfusion pump has been described by Field, de Graaf & Wallis (1958).

RESULTS

Repeated doses of hexamethonium

When repeated intravenous doses of 0.5 mg of hexamethonium bromide were given to anaesthetized rats at intervals which allowed recovery of the blood pressure between each dose, successive doses usually produced less effect. In some cases, the fall in pressure was similar and the rate of recovery became more rapid with successive doses, but, most often, both the fall in pressure and the time for recovery became less (Fig. 1). These results are similar to those observed in the cat by Zaimis (1956) and Mantegazza, Tyler & Zaimis (1958).

Duration of anaesthesia. Since the diminishing effect of repeated doses of hexamethonium might be the result of some degree of recovery of cardiovascular reflex activity associated with a decrease in the depth of anaesthesia with time, the effect of hexamethonium injected early in an experiment (within 1 hr of anaesthesia) was compared with the effect of a first dose of hexamethonium injected when anaesthesia was noticeably lighter. With combined chloralose-urethane anaesthesia, the mean percentage fall in blood pressure produced by the first dose of hexamethonium was

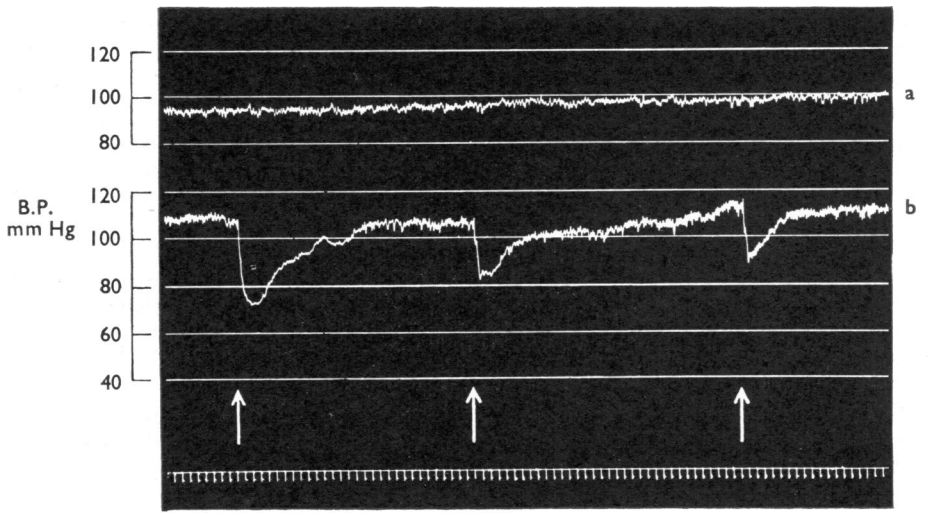

Fig. 1. Two rats, each 280 g, anaesthetized with chloralose-urethane. Simultaneous blood pressure records. (*a*) Control rat. (*b*) Decreasing blood pressure response to successive doses of hexamethonium bromide (0.5 mg) given at arrows. Time in min.

not appreciably different in the two groups. Thus in 7 rats in which 0.5 mg of hexamethonium bromide was first given within 30 to 60 min of anaesthesia, the mean fall of blood pressure was 26% (s.e. 2.6%) compared with 24% (s.e. 3.8%) in 6 rats which had not been given the first dose of hexamethonium till 3 to 4 hr later. In addition, the blood pressure response became less with repeated doses whether the hexamethonium was first given early or late.

Similarly, when chloralose alone was used as anaesthetic, 0.5 mg of hexamethonium bromide given within 30 to 40 min of anaesthesia produced a mean fall of blood pressure in 7 rats of 24% (s.e. 7.1%), which compared with the mean fall of 26% (s.e. 3.2%) observed when 0.5 mg of hexamethonium bromide was given to another 7 rats 1.5 to 2.5 hr after anaesthesia. As with chloralose-urethane anaesthesia, the blood pressure response became less in both groups when the hexamethonium dose was repeated, though it was noted in 3 cases that when the initial fall of blood pressure was small (less than 10%) the diminution of the response was small also.

Hind-limb perfusion experiments

These experiments were designed to estimate the extent to which any local vascular effect of hexamethonium might lead to a decreased blood pressure response to subsequent doses.

In experiments in which the intact hind limb of the rat was perfused at constant rate with its own blood, successive doses of hexamethonium bromide (0.5 mg) produced diminishing blood pressure responses, but in the hind limb the perfusion pressure was reduced by the same proportion each time (Fig. 2). When larger doses of hexamethonium (for example, 0.75 mg) were given, the same effects were observed. However, the recovery of the perfusion pressure after each response was

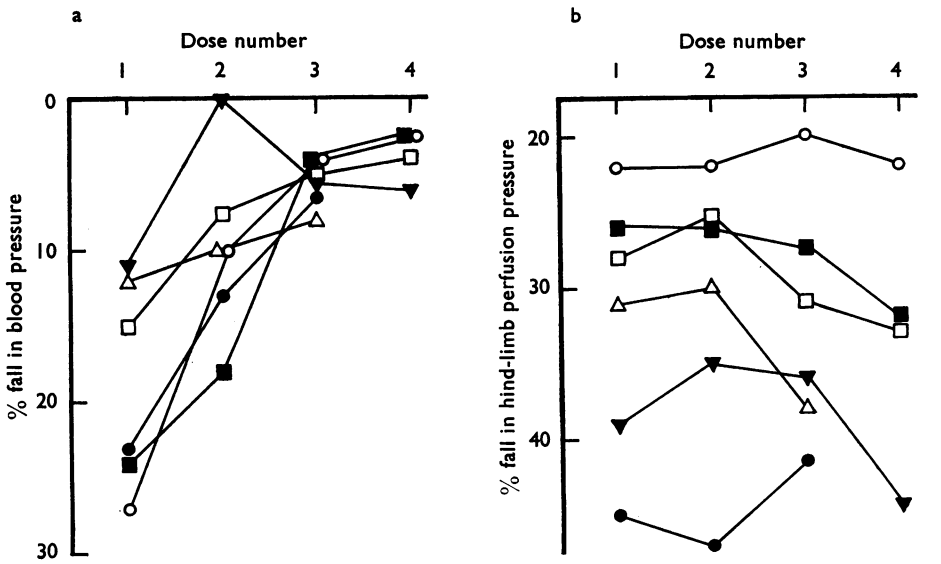

Fig. 2. Changes (*a*) in the blood pressure and (*b*) in the hind-limb perfusion pressure produced by successive doses of hexamethonium bromide (0.5 mg) given to 6 rats. Chloralose anaesthesia. Intact hind limb of each rat perfused with rat's own blood. Doses repeated only when blood pressure had returned to starting level. Results for each rat are distinguished by similar symbols.

less complete so that after several doses of hexamethonium the perfusion pressure was considerably less than at the start of the experiment even though the blood pressure had returned to normal (Fig. 3).

In these experiments, the hind-limb vascular bed was not completely isolated, and the perfusion pressure may have been affected by changes in pressure at the venous outflow or by changes in any collateral circulation present in the hind limb. Further

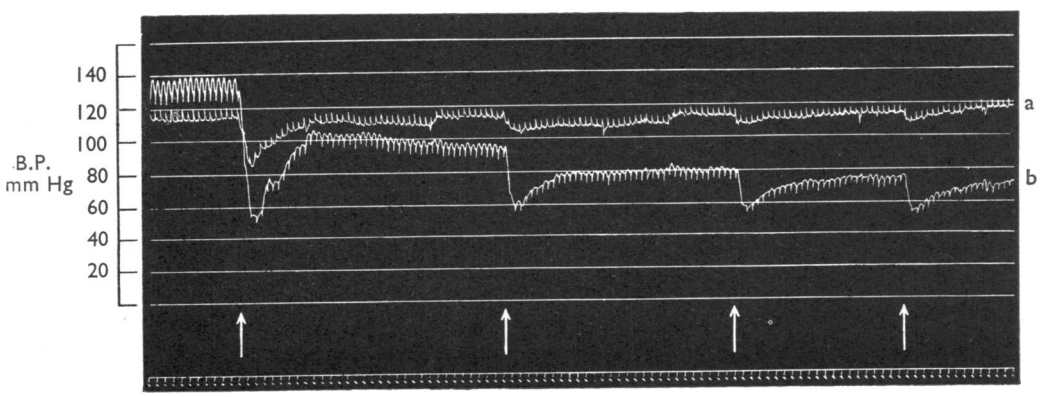

Fig. 3. Rat, 360 g, chloralose anaesthesia. Changes in (*a*) blood pressure and (*b*) hind-limb perfusion pressure produced by successive doses of hexamethonium bromide (0.75 mg) given intravenously at arrows. Hind limb perfused at constant rate with rat's own blood. Note cross-over of blood pressure and perfusion pressure records immediately after first arrow. Time in min.

experiments were therefore performed to see if these factors were important. In three experiments, all vascular connexions except the femoral vein were eliminated. Any collateral circulation was therefore removed. When repeated doses of hexamethonium were given, the changes in blood pressure and hind-limb perfusion pressure followed the same pattern as in the simpler preparation above. In another three experiments, all vascular connexions including the femoral vein were eliminated —only the nerve supply was left intact—and the hind limb was perfused with blood from a separate donor rat. When repeated doses of hexamethonium were given to the recipient animal, the blood pressure and hind-limb perfusion pressure responses showed similar patterns to those in the other experiments. These results suggest that the effects of any collateral circulation in the hind limb or of alterations in pressure at the venous outflow are not important.

Local vascular effects of hexamethonium

The above perfusion experiments suggest that hexamethonium has no local action on blood vessels large enough (at least in the hind limb) to produce a diminishing blood pressure response to repeated doses. However, hexamethonium may still have a small local action.

To obtain more direct evidence the effect of hexamethonium on the vasoconstrictor response to noradrenaline in the isolated hind limb perfused at constant rate with blood from a donor rat was measured. The results are given in Table 1. In the denervated hind limb there was no significant change in the response to noradrenaline

TABLE 1

EFFECT OF HEXAMETHONIUM BROMIDE ON PERFUSION PRESSURE AND VASOCONSTRICTOR RESPONSES TO NORADRENALINE IN THE ISOLATED HIND LIMB OF THE RAT

Test dose of 0.01 μg noradrenaline was added to the blood entering the hind limb. The hexamethonium was usually given to the donor animal. The control values in the last line were obtained in the same way as those of Expt. 2, but hexamethonium was not given

State of preparation	No. of preparations	Dose of hexamethonium (mg)	Mean perfusion pressure (mm Hg)		Mean % rise in perfusion pressure with noradrenaline (0.01 μg)	
			Before hexamethonium	After hexamethonium	Before hexamethonium	After hexamethonium
Denervated	9	3	61·5	63·1	52·0	51·8
Innervated (Expt. no. 1)	14	5	77·9	78·2	46·0	53·4
Innervated (Expt. no. 2)	10	5	112·8	114·4	44·5	50·0
Controls for Expt. no. 2	10	—	109·5	106·6	44·1	48·4

after hexamethonium, but in the innervated hind limb (Expt. 1) there was a significant increase ($0.02>P>0.01$, t test). In a second group of experiments using innervated hind limbs, control responses to noradrenaline were obtained during 40 min before the hexamethonium was given. Over this period there was an increase

in the vasoconstrictor response to noradrenaline similar to that following hexamethonium, and when this was included in an analysis of variance of the results the apparent effect of hexamethonium on the noradrenaline response was not significant.

It should be noted that, in these experiments, hexamethonium had no effect on the perfusion pressure of the hind limb whether innervated or not (Table 1).

DISCUSSION

Our experiments in the rat, like those in the cat reported by Zaimis (1956) and Mantegazza, Tyler & Zaimis (1958), demonstrate that the blood pressure response to repeated doses of hexamethonium becomes less with each dose. We have considered two possible explanations of this effect.

First, a decrease in the depth of anaesthesia during the experiment might allow an increase in cardiovascular reflex activity. This has been shown to be unlikely. The duration of anaesthesia had no influence on the initial blood pressure response to hexamethonium or on the lessening of the response to subsequent doses.

A second explanation has been suggested by Zaimis and her colleagues (Zaimis, 1955, 1956; Mantegazza, Tyler & Zaimis, 1958) on the basis of studies in the cat, namely, that hexamethonium by a local action can sensitize blood vessels to the constrictor effect of noradrenaline sufficiently to mask the effect of its ganglion-blocking action. We have attempted to obtain evidence of this in the rat by perfusing one hind limb at constant rate with the rat's own blood. Because the perfusion was at constant rate, the perfusion pressure should depend only on the combined vasoconstrictor nervous activity and vascular reactivity. Hexamethonium will reduce vasoconstrictor activity by ganglion blockade. It may also enhance vascular reactivity by a local action. If such local action is sufficient to overcome the effects of the ganglion blockade as postulated, the falls in hind-limb perfusion pressure produced by successive doses of hexamethonium should follow the blood pressure responses and become smaller. We did not find this in the rat. With successive doses of hexamethonium the hind-limb perfusion pressure fell by the same proportion each time. Nevertheless, the blood pressure response became less with each dose. This result shows that, at least in the hind limb, hexamethonium does not sensitize blood vessels to noradrenaline enough to mask the effect of its ganglion-blocking action. It is not of course denied that hexamethonium may have a slight local sensitizing action on blood vessels.

In the experiments with larger doses of hexamethonium, the increasing failure of the hind-limb perfusion pressure to recover after each dose no doubt reflects the increasing ganglion blockade resulting from the cumulation of hexamethonium in the circulation. In spite of this evident cumulation, the blood pressure was able to return to normal after each dose of hexamethonium. This is in accord with the observations of Zaimis (1956) in the cat. She found that the response of the nictitating membrane to preganglionic stimulation was increasingly reduced by successive doses of hexamethonium while the blood pressure response became less.

It is possible that changes in pressure at the venous outflow of the hind limb or in any collateral circulation in the hind limb occurred in our experiments so that

the hind-limb perfusion pressure was not simply dependent on the vasoconstrictor nervous activity and vascular reactivity as we have assumed. However, when the rat was prepared so that any collateral circulation was removed, the results were the same as in the simpler preparation. Even when the hind limb was completely isolated except for its nervous connexions and perfused with blood from a donor rat, the results were essentially similar. In this case there was no hexamethonium circulating in the hind limb. It would appear then that any pressure changes at the venous outflow or in any collateral circulation in the hind limb are not important.

Our experiments with the isolated hind-limb preparation suggest that any effect of hexamethonium on the response of the hind-limb blood vessels to noradrenaline is small. Statistically significant effects were only observed when the innervated hind-limb preparations were used without adequate control experiments. The fact that the level of perfusion pressure did not increase when hexamethonium was added to the blood perfusing the innervated hind limb supports the conclusions that any local action of hexamethonium is small. Any large local action would be expected to enhance the effect of the noradrenaline released endogenously by the vasoconstrictor nerves.

From our results we cannot say why the blood pressure response to repeated doses of hexamethonium becomes less with each dose in the anaesthetized rat. It is clear that local effects of hexamethonium on the blood vessels of the hind limb are not sufficient to mask the ganglion-blocking effects. Evidently the changes which reduce the blood pressure response occur elsewhere in the cardiovascular system, presumably in the heart or possibly in blood vessels other than in the hind limb.

The salary of one of us (R. L.) was provided by the Life Insurance Medical Research Fund of Australia and New Zealand. We also acknowledge financial assistance from the Medical Research Council of New Zealand.

REFERENCES

FIELD, L. W., DE GRAAF, W. & WALLIS, A. T. (1958). Constant output blood perfusion pump for use in cross-transfusion experiments in small laboratory animals. *J. appl. Physiol.*, **12**, 142–144.

FIELD, L. W. & LAVERTY, R. (1958). Nervous and humoral responses to acute blood loss in the rat. *J. Physiol. (Lond.)*, **143**, 213–225.

MANTEGAZZA, P., TYLER, C. & ZAIMIS, E. (1958). The peripheral action of hexamethonium and pentolinium. *Brit. J. Pharmacol.*, **13**, 480–484.

ZAIMIS, E. J. (1955). The interruption of ganglionic transmission and some of its problems. *J. Pharm. (Lond.)*, **7**, 497–511.

ZAIMIS, E. J. (1956). A possible explanation for the development of "tolerance" to ganglion-blocking substances. In *Hypotensive Drugs*, pp. 85–92, ed. M. HARINGTON. London: Pergamon Press.