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ON THE MECHANISM OF THE ANTI-INFLAMMATORY ACTIVITY OF HEXADIMETHRINE BROMIDE

BY

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Hexadimethrine bromide, an antiheparin agent, prevents the activation of plasma kinins in plasma or serum that has been subjected to a variety of *in vitro* procedures (Armstrong & Stewart, 1962). It shows anti-inflammatory activity in the cotton-pellet and rat-paw oedema tests, and inhibits the tuberculin response in B.C.G.-sensitized guinea-pigs (Kellett, 1965). Since kinins are probably involved in inflammatory processes (Lewis, 1960), it was originally thought that the anti-inflammatory effects might result from inhibition of kinin formation. However, the large doses of drug needed to inhibit oedema formation in the rat-paw test in normal animals, and the reduction of activity in adrenalectomized animals, rendered this hypothesis untenable. A detailed investigation was therefore made into the mechanism of the drug's anti-inflammatory activity.

METHODS

In vitro *inhibition by hexadimethrine of kinin formation in serum*

Rats and guinea-pigs were stunned by a blow on the head. Blood was collected from a jugular vein into polyethylene tubes and allowed to clot. Serum from these blood samples was diluted 1:30 with Tyrode solution containing atropine (0.01 μg/ml.) and mepyramine (0.02 μg/ml.) and incubated at 37° C for 3 min. Hexadimethrine was added to the diluted serum either before incubation or immediately before testing for kinin content. Kinin content was determined on the guinea-pig ileum preparation in a 3 ml. organ-bath at 37° C, using Tyrode solution containing atropine and mepyramine as described above. Usually 0.1 ml. of serum was used to elicit kinin-induced contractions of the ileum, and when added to the bath this dose produced a relatively slow contraction. When diluted serum was tested, 0.1 ml. of serum was incubated with 2.9 ml. of Tyrode solution, as described previously, and this incubation mixture was poured into the previously emptied bath.

Effect of hexadimethrine on bradykininogen levels after retarded anaphylaxis in guinea-pigs

Male albino guinea-pigs were sensitized by the intramuscular and intraperitoneal injection of 100 mg of twice crystallized egg albumin (Sigma Chemicals). Four weeks later the animals were given saline or hexadimethrine (7.5 mg/kg) intraperitoneally 30 min before the injection of 0.5 ml. of alum-precipitated antigen (50 mg of egg albumin) by the same route. Blood was obtained by cardiac puncture before the injection of saline or of hexadimethrine, immediately before the antigen, and again 4 hr later. Heparin (5 U/ml.) was added to the blood, which was stored in ice-cold water. The plasma was separated by centrifugation at 5° C and a 1 ml. sample was heated in a boiling-water

bath for 3 min. After further centrifugation the residue was incubated with 1 ml. of trypsin (0.5 mg/ml.) at 37° C for 30 min.

The supernatant fluid from this incubation mixture was assayed for kinin content against synthetic bradykinin on the isolated preparation of guinea-pig ileum.

Effect of adrenalectomy and removal of the adrenal medullae on the anti-oedematous activity of hexadimethrine

Rats were adrenalectomized by the dorsal route during ether anaesthesia. Removal of the adrenal medullae was carried out as follows: the rat was anaesthetized with ether and one adrenal gland was exposed, as for adrenalectomy, using the dorsal approach, and taking care to avoid damaging the vascular supply to the gland. A small incision was made in the cortex; slight pressure applied to the base of the gland was sufficient to eject the medulla. The medulla of the other adrenal gland was removed in the same way and the skin wound was closed with suture clips. One week was allowed for recovery from either operative procedure. With the adrenalectomized rats drinking-water was replaced by 1% saline solution.

Anti-oedematous activity was estimated by the rat-paw test, as described previously (Kellett, 1965), 0.1 ml. of supernatant fluid from a 10 or 20% yeast suspension being used as irritant. Ether was normally used to immobilize the animals for the foot-volume measurement, but when a blood sample was also to be collected carbon dioxide was used as anaesthetic. Drugs were given at the following times before the yeast injection: hexadimethrine (intraperitoneally) 30 min; adrenaline (subcutaneously) 30 min; corticotrophin (intramuscularly) 24 hr and 2 hr; cyproheptadine (intraperitoneally) 30 min; propranolol (intramuscularly) as described; and mecamylamine (subcutaneously) 45 min.

Effect of hexadimethrine on the blood-sugar levels of normal, adrenalectomized and splanchnic denervated cats

Anaesthesia was induced with ether and maintained with chloralose (100 mg/kg, intravenously). Drugs were injected through a cannula in a femoral vein and blood was collected from a carotid artery. A tracheal cannula was inserted. After adrenalectomy, splanchnic denervation, or mock adrenalectomy, the animals were left for 2 hr before the collection of blood samples. Samples were obtained at 15 min intervals from 1 hr before dosing with 10 mg/kg of hexadimethrine until 2 hr afterwards.

Blood-sugar estimations

Blood-sugar concentrations were estimated by the ferricyanide method, using the Autoanalyser (Technicon). Unless otherwise stated blood was collected by cardiac puncture during anaesthesia with carbon dioxide.

Drugs

These were: hexadimethrine bromide (Abbott); adrenaline hydrochloride (B.D.H.); corticotrophin (Cortotrophin Zn, Organon); propranolol (I.C.I.); mecamylamine (May & Baker); cyproheptadine (Merck, Sharpe and Dohme); and bradykinin (Sandoz).

RESULTS

Effect of hexadimethrine on formation of kinins in vitro

The *in vitro* inhibition by hexadimethrine of kinin formation after dilution of serum (Armstrong & Stewart, 1962) was confirmed (Fig. 1). Hexadimethrine (5 μg/ml.) almost completely prevented the formation of kinin in serum both from rats and from guinea-pigs.

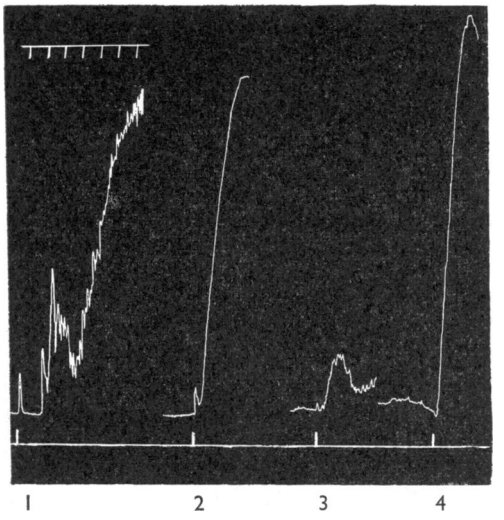

Fig. 1. Inhibition, by hexadimethrine, of kinin formation in guinea-pig serum. Guinea-pig ileum preparation in Tyrode solution containing atropine (0.01 μg/ml.) and mepyramine (0.02 μg/ml.). Bath temperature, 32° C. Bath volume, 3 ml. Time marks, 30 sec. At 1, 0.1 ml. of serum was added to the bath; at 2, 0.1 ml. of serum incubated for 3 min at 37° C with 2.9 ml. of Tyrode solution (plus atropine and mepyramine); at 3, as 2, but hexadimethrine (5 μg/ml.) was added before incubation; at 4, as 2, but hexadimethrine (5 μg/ml.) was added after incubation.

Hexadimethrine and retarded anaphylaxis

Bradykinin is released during anaphylactic shock in rats and guinea-pigs, with a resultant decrease in the levels of serum bradykininogen (Brocklehurst & Lahiri, 1962). If hexadimethrine prevents kinin activation the levels of bradykininogen should not be altered in hexadimethrine-treated animals after retarded anaphylaxis. The results of experiments in guinea-pigs, summarized in Table 1, show that the serum levels of bradykininogen, as estimated by the release of bradykinin after incubation with trypsin, were significantly reduced in control and hexadimethrine-treated groups after retarded anaphylaxis. There appeared to be rather less depletion in animals which had received hexadimethrine, but the difference between the two groups was not statistically significant.

TABLE 1

EFFECT OF HEXADIMETHRINE ON BRADYKININOGEN LEVELS IN BLOOD OF SENSITIZED GUINEA-PIGS BEFORE AND AFTER RETARDED ANAPHYLAXIS

Blood samples were taken before treatment by intraperitoneal injection of 0·75 ml. of saline or 7·5 mg/kg of hexadimethrine, immediately prior to challenge with antigen and 3 hr after challenge. The results are expressed as μg/ml. of bradykinin released from serum after incubation with 0·5 mg/ml. of trypsin. Results are means and standard errors

Treatment	No. of animals	Blood bradykinin (μg/ml.) Before treatment	Before challenge	After challenge	Reduction in bradykinin due to challenge (%)
Saline	9	9·8±0·7	10·52±0·7	6·22±0·4	40·9±4·13
Hexadimethrine	8	9·4±1·2	9·3 ±0·5	6·34±0·9	31·8±5·14

Anti-inflammatory activity of hexadimethrine in adrenalectomized rats and in rats with adrenal medullae removed

In adrenalectomized rats the anti-oedematous activity of hexadimethrine (40 mg/kg) was approximately one-quarter of that seen in normal animals (Table 2). Absence of the adrenal cortex was unlikely to be responsible for this loss of activity, since large doses of corticotrophin, given to intact rats, had very little anti-inflammatory effect.

TABLE 2

ANTI-OEDEMATOUS AND HYPERGLYCAEMIC ACTIVITIES OF HEXADIMETHRINE IN NORMAL AND ADRENALECTOMIZED RATS AND IN RATS WITH ADRENAL MEDULLAE REMOVED, AND ANTI-OEDEMATOUS ACTIVITY OF CORTICOTROPHIN IN NORMAL RATS

Oedema was produced by injecting 0·1 ml. of the supernatant fluid from a 20% yeast suspension beneath the left hind-paw. Corticotrophin (20 U/kg, subcutaneously) was given 24 and 2 hr, and hexadimethrine (40 mg/kg, intraperitoneally) was given 30 min before the yeast injection. The results refer to foot volume measurements taken 45 min after injection of irritant. There were six rats per group. Results are means and standard errors

Condition	Treatment	Increase in foot volume (%)	Inhibition of oedema (%)	Blood sugar (mg/100 ml.)
Normal	Saline	81·7±4·3	—	89±3·6
	Hexadimethrine	20·6±3·9	74·8	147±7·5
	Corticotrophin	69·7±2·6	14·7	—
Adrenalectomized	Saline	84·5±4·1	—	71±2·8
	Hexadimethrine	69·5±5·2	17·8	74±4·6
Adrenal medullae removed	Saline	71·4±2·1	—	84±3·1
	Hexadimethrine	62·0±3·7	13·2	91±3·4

It was possible, however, that hexadimethrine released more, or different, steroids from the cortex. The dependence of hexadimethrine on the adrenal medulla for its activity was shown in experiments using animals with adrenal medullae removed (Table 2). In these animals the anti-oedematous activity of hexadimethrine was usually about 10% of that seen in normal animals but occasionally the drug retained up to 25% of its activity.

Hexadimethrine, injected intravenously or intraperitoneally, produced a marked hyperglycaemic response which, in rats, could be abolished not only by adrenalectomy but also by removal of the adrenal medullae (Table 2). We showed that in cats the drug acted directly on the adrenal medulla. In normal or splanchnic-denervated cats hyperglycaemia developed rapidly after the intravenous injection of 10 mg/kg of hexadimethrine, but the drug had no effect in adrenalectomized animals (Table 3).

TABLE 3

EFFECT OF HEXADIMETHRINE ON THE BLOOD-SUGAR LEVELS OF NORMAL, SPLANCHNIC DENERVATED AND ADRENALECTOMIZED CATS

Hexadimethrine (10 mg/kg) was injected into each cat at zero time. Each result is the mean of two experiments

Time (min)	Blood sugar (mg/100 ml.)			
	Control	Mock adrenalectomized	Adrenalectomized	Splanchnic denervated
−60	105	115	67	89
−30	101	104	72	94
0	104	94	70	103
30	215	193	86	224
60	304	287	74	321
90	240	254	76	265
120	190	239	79	228

In rats blockade of ganglionic transmission by 40 mg/kg of mecamylamine (subcutaneously) did not affect either the anti-oedematous or the hyperglycaemic effects of hexadimethrine (Table 4), which suggests that the antiheparin drug acts directly on the medullary chromaffin cells.

TABLE 4

ANTI-OEDEMATOUS AND HYPERGLYCAEMIC EFFECTS OF ADRENALINE AND HEXADIMETHRINE IN MECAMYLAMINE-TREATED RATS

Oedema was produced by injecting 0·1 ml. of the supernatant fluid from a 10% yeast suspension beneath the left hind-paw. Mecamylamine (20 mg/kg, subcutaneously) was given 45 min, and adrenaline (0·5 mg/kg, subcutaneously) and hexadimethrine (30 mg/kg, intraperitoneally) were given 30 min before the injection of irritant. The results refer to foot volume measurements taken 45 min after the yeast injection. There were six rats per group. Results are means and standard errors

Treatment	Increase in foot volume (%)	Inhibition of oedema (%)	Blood sugar (mg/100 ml.)
Saline	75·9±4·7	—	93·2±3·9
Adrenaline	20·2±1·8	73·4	176·0±8·2
Hexadimethrine	33·0±2·7	56·5	149·7±7·4
Mecamylamine	66·6±5·0	12·3	86·0±2·8
Adrenaline+mecamylamine	26·1±3·6	65·6	189·6±5·0
Hexadimethrine+mecamylamine	35·6±4·2	53·1	150·0±6·7

Propranolol (10 mg/kg) was used to antagonize the anti-oedematous effect of adrenaline. However, the results summarized in Table 5 show that the same dose of propranolol did not reduce the anti-oedematous effect of hexadimethrine; nor did repeated doses of propranolol. The anti-oedematous effect of the 5-hydroxytryptamine antagonist cyproheptadine also remained unaffected after propranolol.

TABLE 5

EFFECT OF PROPRANOLOL ON THE ANTI-OEDEMATOUS ACTIVITY OF ADRENALINE HEXADIMETHRINE AND CYPROHEPTADINE

Oedema was produced by injecting 0·1 ml. of the supernatant fluid from a 10% yeast suspension beneath the left hind-paw. Drugs were given at the following times before the yeast injection: propranolol (10 mg/kg, intramuscularly) as a single dose 40 min or in two doses (when indicated) 40 and 10 min; adrenaline (0·5 mg/kg, subcutaneously) 30 min; hexadimethrine (30 mg/kg, intraperitoneally) 30 min; and cyproheptadine (2 mg/kg, intraperitoneally) 30 min. The results refer to foot volume measurements taken 45 min after the injection of irritant. There were six rats per group

Treatment	Inhibition of oedema (%)
Adrenaline	77·2
Hexadimethrine	51·6
Cyproheptadine	63·8
Propranolol	0
Adrenaline+propranolol	23·4
Hexadimethrine+propranolol	54·6
Hexadimethrine+propranolol (two doses)	57·4
Cyproheptadine+propranolol	64·4

DISCUSSION

Hexadimethrine has anti-inflammatory activity in a variety of tests (Eisen, 1964; Brown & Robson, 1964; Kellett, 1965), and this has been attributed to inhibition of kinin formation. The drug prevents the activation of kinins induced in plasma and serum by various procedures *in vitro* (Armstrong & Stewart, 1962), and this has been confirmed in the present experiments.

However, the drug did not appear to prevent kinin activation during anaphylaxis *in vivo*. There was no significant difference between the reduction in bradykininogen levels in sera from saline- and hexadimethrine-treated guinea-pigs after retarded anaphylaxis, although a significant difference might have been revealed by use of larger groups of animals.

From the results of experiments with rats with adrenal medullae removed it seems that, in the rat-paw test at least, the anti-oedematous activity of hexadimethrine is largely, but not completely, mediated by a release of catechol amines from the medulla. Experiments on splanchnic-denervated cats, and on rats in which ganglionic transmission had been blocked, suggest that this release of catechol amines is due to a direct action of hexadimethrine on the medullary chromaffin cells.

Inhibition of the anti-oedematous effect of adrenaline was achieved with large doses of propranolol, but we have so far been unable to modify the response to hexadimethrine with this antagonist. It is questionable whether these results are significant in view of the large dose (10 mg/kg) of antagonist used. But in unpublished experiments we have shown that the ratio of activities of propranolol and pronethalol, as inhibitors of the anti-oedematous activity of adrenaline, corresponds closely with the clinical and experimental potencies of these compounds when tested as sympathetic β-receptor antagonists (Black, Crowther, Shanks, Smith & Dornhorst, 1964). Propranolol did not affect the inhibition of a yeast oedema by cyproheptadine, and the same is true for antagonism of the inhibition, by hydrocortisone, phenylbutazone and indomethacin, of a carrageenin-induced oedema (unpublished observations).

It should be noted that, whereas adrenalectomy or removal of the adrenal medullae in rats and adrenalectomy in cats completely inhibited the hyperglycaemic response to hexadimethrine, removal of the adrenal medullae in rats resulted in only partial inhibition of its anti-oedematous activity. One-quarter to one-tenth of the anti-oedematous effect of the drug remained after removal of the adrenal medullae and this may be a reflection of the effect of hexadimethrine on kinin-activation.

Whether the activity of hexadimethrine in other anti-inflammatory tests depends on catechol amine release is being investigated.

SUMMARY

1. Hexadimethrine prevented the activation of kinins after dilution of serum *in vitro*, but did not significantly affect depletion of bradykininogen during retarded anaphylaxis *in vivo*.

2. Adrenalectomy or removal of the adrenal medullae in rats significantly reduced the anti-oedematous activity of hexadimethrine. Its hyperglycaemic effect was absent in rats with adrenal medullae removed.

3. Release of catechol amines, by a direct action of hexadimethrine on the medullary cells, is probably mainly responsible for the the drug's anti-oedematous activity.

4. Propranolol antagonized the anti-oedematous activity of adrenaline but did not affect the response to hexadimethrine.

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