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EFFECTS OF SOME *ISO*THIOUREA AND GUANIDINE SALTS ON VARIOUS PREPARATIONS OF SMOOTH AND STRIPED MUSCLE

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In seeking pressor agents of new types, Smirk (1941) was led to test the fairly well known organic chemical methyl *iso*thiourea sulphate. He found it capable of producing large persistent rises of blood pressure in anaesthetized animals. According to McGeorge, Sherif, and Smirk (1942), the rises of blood pressure are brought about mainly if not entirely through a direct action of methyl *iso*thiourea on blood vessels; and this action is not sympathomimetic. They state that the pressor action was hardly affected by the anti-adrenaline compounds, ergotoxine and F933. Methyl *iso*-thiourea, unlike adrenaline, tended to increase the tone of isolated muscle strips from rabbit intestine, bladder, and uterus.

Certain of its chemical relatives have been found to behave similarly: the pharmacological properties of methyl *iso*thiourea are reproduced with most fidelity by amidine derivatives like ethyl *iso*urea and guanidine salts which ionize freely to yield cations of small size (Fastier and Smirk, 1943 and 1947; Fastier, 1948). It would appear that pressor analogues of methyl *iso*thiourea need have nothing more in common with it *structurally* than the amidine group

$$\left(-C\langle^{NH-}_{NH-}\right)^{+}$$

Now histamine contains this group. Moreover, it is known that histamine too causes the contraction of most kinds of smooth muscle irrespective of the innervation. The possibility that amidine derivatives like methyl *iso*thiourea and guanidine produce some of their more characteristic pharmacological effects through simulating actions of histamine has therefore appeared worthy of investigation.

Other experiments reported below were suggested by a much older idea—one which goes back to Führer (1908), who compared the action of guanidine on striped muscle to that of a univalent alkali metal cation. He pointed out that the effects of both are antagonized by such divalent cations as calcium. Similar observations on the interaction of calcium and guanidine have been published by a number of workers (Major and Stephenson, 1924; Ochoa, 1928; Minot, 1931; Burns and Secker, 1935; Harvey, 1940). Minot, Dodd, and Riven (1938) express what is probably a widely held view when they write: "It is possible that guanidine exerts its action on muscles through changes in the effect of inorganic salts." For this reason, the effect of changes in calcium or in potassium ion concentration on the response to typical amidine derivatives has also been studied.

METHODS

Rabbits' ears were perfused at room temperature with Ringer-Locke solution kept at a constant head of pressure by means of a Mariotte bottle (Kravkov-Pissemski preparation). The venous outflow was measured by the method of Stephenson (1948). Amidine derivatives were injected as salts in neutral solution. The *iso*thioureas tested were some of those specially synthesized for this series of investigations. Samples of guanidine hydrochloride, methylguanidine sulphate, and *asym*-dimethylguanidine sulphate were obtained from commercial sources, as were also such drugs as "Priscol" and *d*-tubocurarine chloride.

Isolated strips of rabbit or guinea-pig ileum and uterine horns of non-pregnant rats were set up in an ordinary organ bath of 100 ml. capacity. The Ringer-Tyrode solution in the bath was aerated with oxygen containing 5 per cent carbon dioxide.

The bronchiole preparation employed was that of Konzett and Rössler (1940). Female guinea-pigs of 500–600 g. weight were deeply anaesthetized by injecting urethane intraperitoneally. Cannulae were inserted into a jugular vein and the trachea. The lungs were insufflated with a small Starling respiration pump

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at each stroke of which the excess air from the pump was driven into a float recorder. When the bronchioles were constricted the average level of the marker was raised (see Fig. 3).

Rectus abdominis muscle was taken from winter frogs (*Rana temporaria*). Each piece was suspended in a 5 ml. bath through which air was bubbled slowly. Isotonic contractions were recorded with a gimbal lever. The Ringer solution used contained 0.7 g. NaCl, 0.014 g. KCl, 0.012 g. $CaCl_2$, and 0.02 g. $NaHCO_3$ per 100 ml. Acetylcholine was added in known concentration (usually a 1 in 2,000,000 solution), replacing the ordinary Ringer solution of the bath for a period of 90 seconds. The muscle strip was washed two or three times after each dose of acetylcholine. The interval between doses was kept at 5 minutes.

The rat diaphragm preparation was set up as specified by Bülbring (1946). At first the bath temperature was kept at 39° C., but later a temperature of 28° C. was chosen as more suitable. Drug solutions were added directly to the bath. A "square wave" stimulator was employed; its construction permitted the amplitude, duration, and frequency of the shocks to be varied independently. The nerve was stimulated in the fluid from platinum contacts.

Experiments on cat gastrocnemius muscle were performed on animals under chloralose anaesthesia. The muscle was stimulated via the sciatic nerve with condenser shocks strong enough to give a maximal response. Contractions were recorded by attaching a stout wire from an isometric lever which wrote on a slowly moving drum to the lower end of the tendo Achillis. The central attachment of the muscle had previously been fixed firmly in position by clamping a steel rod driven through the condyles of the left femur. Drug solutions were forced into the left iliac artery through a cannula whose tip lay just below the bifurcation of the aorta.

RESULTS

Effects on smooth muscle preparations

Perfused rabbit ear

Methyl *iso*thiourea was tested on eighteen perfused ears. A dose of 0.1 ml. of an M/5,000 solution brought about definite vasoconstriction (Fig. 1*b*) in six of eight experiments. Strong vasoconstriction was caused in all of seventeen ears by injecting 0.1 ml. of an M/1,000 solution. When 0.1 ml. of an M/100 solution was injected, its effect was intense.

Smaller vasconstrictor effects were obtained as a rule when *asym*-dimethylguanidine was given in corresponding amount. Guanidine itself was less active still.

Effect of "Priscol." — In ten experiments benzyliminazoline ("Priscol") hydrochloride was perfused as a 1 : 5,000 solution in Ringer-Locke after control injections of methyl *iso*thiourea and of adrenaline had been given. Within a few minutes the response to even a large (0.1 μg.) dose of adrenaline was "reversed." Nevertheless, vasoconstriction was still brought about when methyl *iso*thiourea was injected (Fig. 1*d*). Its action did not seem to be affected appreciably by the priscol; nor did that of guanidine or of *asym*-dimethylguanidine.

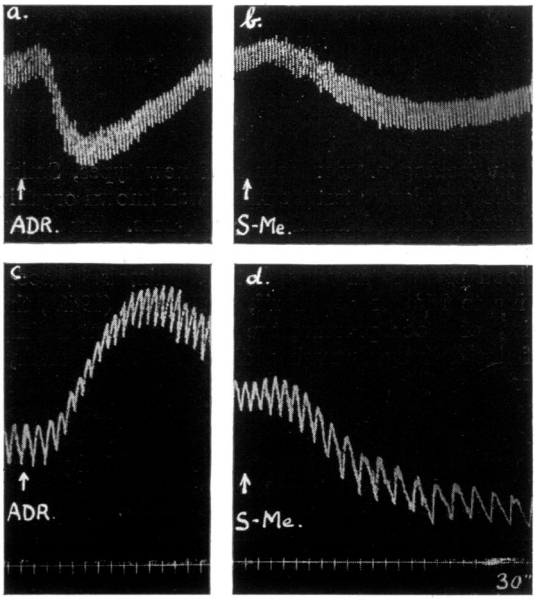

FIG. 1.—Perfused rabbit ear preparation. Upper tracings show the normal effect of (*a*) adrenaline (0.01 μg. at ADR) and (*b*) methyl *iso*thiourea (0.1 ml. of M/5,000 at S-Me). Lower tracings show the effect of (*c*) adrenaline (0.1 μg. at ADR) and (*d*) methyl *iso*thiourea (0.1 ml. of M/5,000 at S-Me) after a 1 : 5,000 solution of priscol has been perfused for about ten minutes.

Effect of "Anthisan."—Unlike priscol, the antihistamine compound N - *p* - methoxybenzyl - N - dimethylaminoethyl - α - aminopyridine ("Anthisan") maleate antagonized the vasoconstrictor response to methyl *iso*thiourea in concentrations which left the blood vessels still fairly responsive to adrenaline. In all of seven experiments it soon diminished the response to a test dose of methyl *iso*thiourea when perfused in M/1,000 solution (Fig. 2). Ultimately even so large a dose as 0.1 ml. of M/10 methyl *iso*thiourea failed to produce vasoconstriction. Slight vasodilatation was obtained occasionally with the *iso*thiourea, but there was nothing comparable to the "reversal" seen with adrenaline after priscol.

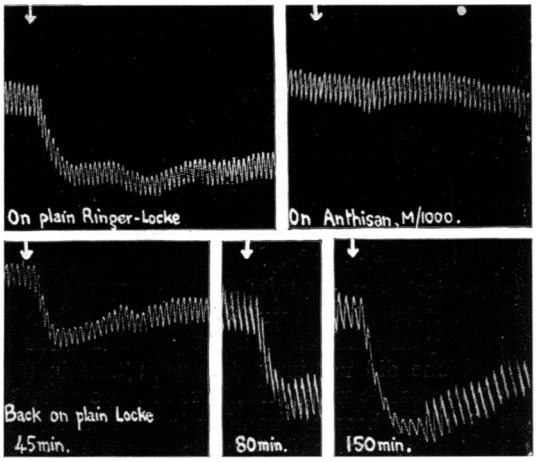

FIG. 2.—Perfused rabbit ear preparation. Methyl *iso*thiourea sulphate (0.1 ml. of M/100 given at the arrows). Its effect is practically suppressed when anthisan (M/1,000) is added to the perfusing Ringer-Locke solution. Even a very large dose of methyl *iso*thiourea (0.1 ml. of M/10 at the dot) produces hardly any vasoconstriction.

Anthisan in this amount was found to antagonize the vasoconstrictor action of adrenaline too, although not so completely; vasoconstriction could still be produced in response to a large (2 μg.) dose of adrenaline at a time when the effect of even a 1 mg. dose of methyl *iso*thiourea was quite suppressed. Sensitivity to methyl *iso*thiourea was very slowly restored when ordinary Ringer-Locke solution was perfused again in place of the anthisan solution.

Isolated gut

Slight but definite antagonism between anthisan and methyl *iso*thiourea was also noticed in organ bath experiments with guinea-pig ileum. Methyl *iso*thiourea tends to increase the tonus of the strip when it is added to make a bath concentration of M/10,000 or higher. This effect was found to be suppressed more or less completely when anthisan was given before the *iso*thiourea in concentrations of M/10,000 and upwards.

The tonus-increasing action of methyl *iso*thiourea on isolated rabbit ileum was not much impaired by a concentration of atropine (M/10,000) sufficient to render the muscle insensitive to even large amounts of acetylcholine. When guinea-pig ileum was used in place of rabbit ileum, however, it was found that this same concentration of atropine greatly reduced the response to a test dose of methyl *iso*thiourea.

In the presence of methyl *iso*thiourea the response of guinea-pig ileum to a test dose of histamine may be increased or decreased, according to the conditions employed. A decrease was always obtained when the dose of *iso*thiourea was of itself sufficient to produce a considerable rise in tone. Higher homologues like *n*-butyl and *n*-heptyl *iso*thiourea were found to exert a much stronger spasmolytic action, dose for dose. A slightly increased response to a small dose of histamine was seen occasionally with slightly smaller concentrations of methyl *iso*thiourea (of the order of M/10,000). Potentiation of the response to histamine by methyl *iso*thiourea was observed quite regularly when the strip had first been rendered moderately insensitive to histamine by treatment with anthisan. In these circumstances, doses of methyl *iso*thiourea or of *asym*-dimethylguanidine which had little effect themselves on muscle tone increased appreciably the response to a test dose of histamine.

TABLE I

EFFECTS OF *iso*THIOUREAS UPON THE BRONCHOCONSTRICTOR RESPONSE TO HISTAMINE

Substance	Dose	No. of exps. in which histamine response was		
		Increased	Unchanged	Decreased
Methyl *iso*thiourea	0.1 ml. M/10	5	4	
,, ,, ...	0.25 ml. ,,	4	4	
n-Hexyl ,, ...	0.1 ml. ,,			3
,, ,, ...	0.25 ml. ,,			1
n-Heptyl ,, ...	0.1 ml. ,,		1	2
,, ,, ...	0.25 ml. ,,			3
Tetramethylene di-*iso*thiourea ...	0.1 ml. M/20	1	2	
,, ,, ...	0.25 ml. ,,		1	2

Guinea-pig lung preparation

Slight transitory bronchoconstrictor responses were produced in the following experiments by injecting small test doses of histamine acid phosphate (2–10 μg./kg.) at regular intervals of five minutes or more. The response to histamine was altered by various *iso*thioureas as indicated in Table I. In about half the experiments performed with methyl *iso*thiourea the bronchoconstrictor effect of histamine was definitely potentiated (Fig. 3); it was never reduced. However, higher

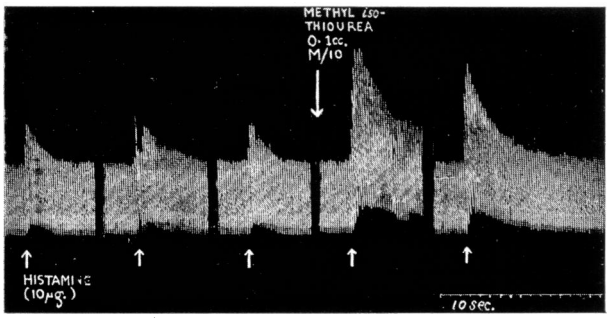

FIG. 3.—Guinea-pig lung preparation. The bronchoconstrictor effects were produced by 10 μg. doses of histamine acid phosphate injected at 10 min. intervals. The response to histamine is increased after a dose of methyl *iso*thiourea (0.1 ml. of an M/10 aqueous solution of the sulphate).

homologues usually produced anti-histamine effects when given in the same range of doses. Sometimes the effect of histamine was reduced by as much as 60–80 per cent.

Methyl *iso*thiourea had no distinctive effect *per se*; exceptionally it caused slight bronchoconstriction. In two of the experiments with *n*-heptyl *iso*thiourea there was considerable bronchoconstriction after its injection. Unfortunately the interpretation of some of these results was made difficult because spontaneous respiratory movements were resumed soon after the *iso*thiourea was injected. In most instances the compounds had no direct effect on the bronchioles.

Isolated rat uterus

It was confirmed that histamine has an inhibitory action on non-pregnant rat uterus. It should be added, however, that doses of the order of 1–5 μg./ml. were required to demonstrate any effect, although the horns were quite sensitive to adrenaline and acetylcholine.

Methyl *iso*thiourea was tested several times on each of eight preparations in concentrations of from M/10,000 to M/2,000. Usually it had no effect at all; sometimes there appeared to be slight stimulation. More definite evidence of stimulation was obtained with *n*-butyl *iso*thiourea. In four of six experiments a bath concentration of M/2,000 caused a slight increase in the tone and motility of the strip.

Effects on striped muscle preparations

Isolated frog rectus abdominis muscle

In ten successive experiments the addition of methyl *iso*thiourea (M/500) to the test dose of acetylcholine resulted in an enhanced response of the muscle. The *iso*thiourea alone did not increase muscle tone in four control experiments, but its potentiating activity became manifest in these too when the Ringer solution containing methyl *iso*thiourea sulphate was replaced by the test solution of acetylcholine.

Potentiation of the response to acetylcholine was also observed regularly in experiments with ethyl *iso*thiourea and with *asym*-dimethylguanidine. On the other hand, the long-chain amidine derivative *n*-hexyl *iso*thiourea was found to antagonize the action of acetylcholine even in a dilution of M/5,000.

Rat phrenic nerve-diaphragm preparation

An enhanced response to a short-acting (0.5 msec.) electrical stimulus—a slightly supermaximal shock given indirectly every 10 seconds—was obtained regularly after the injection of an M/10 solution of one of the following salts into the 100 ml. bath: guanidine hydrochloride (0.5–2.0 ml.), methylguanidine sulphate (0.5–1.0 ml.), methyl *iso*thiourea hydrochloride and sulphate (0.25–1.0 ml.), and ethyl *iso*thiourea hydrobromide (0.25–0.5 ml.). The phenomenon was seen almost immediately after the addition of methyl or ethyl *iso*thiourea, but not for some minutes after the addition of guanidine or of *asym*-dimethylguanidine.

Potentiation after methyl *iso*thiourea was still observed in experiments in which a longer-acting electrical stimulus (35 msec.—the maximum for the instrument) was applied or in which the rate of stimulation was ten times faster.

The higher homologues *n*-butyl and *n*-hexyl *iso*thiourea reduced the amplitude of the twitch when added in the same range of doses as methyl *iso*thiourea. Transitional behaviour was displayed by *n*-propyl *iso*thiourea: whereas moderate concentrations (M/2,000–M/500) somewhat increased the amplitude of the twitch, higher concentrations decreased it.

Interaction with d-tubocurarine.—A dose of 1 μg./ml. of *d*-tubocurarine chloride was found sufficient to curarize the preparation; the muscle could now be made to contract only by stimulating it directly. Given at this point guanidine (M/500) increased considerably the contractions brought about by direct stimulation, but indirect stimulation indicated that this potentiating effect of guanidine was due in part to an anti-curare action; such an action was demonstrated directly in six experiments. Methyl *iso*thiourea was found to behave similarly when given in a concentration of M/4,000. Its potentiating action was seen best when the dose of *d*-tubocurarine administered was one which produced only partial block.

When the dose of *d*-tubocurarine was increased to 2 μg./ml., no anti-curare action could be demonstrated with the above doses of methyl *iso*thiourea and guanidine. Nevertheless, both amidine derivatives tended to augment the effect of direct electrical stimuli if given under these conditions; methyl *iso*thiourea to nearly its usual extent but guanidine sometimes hardly at all (Fig. 4).

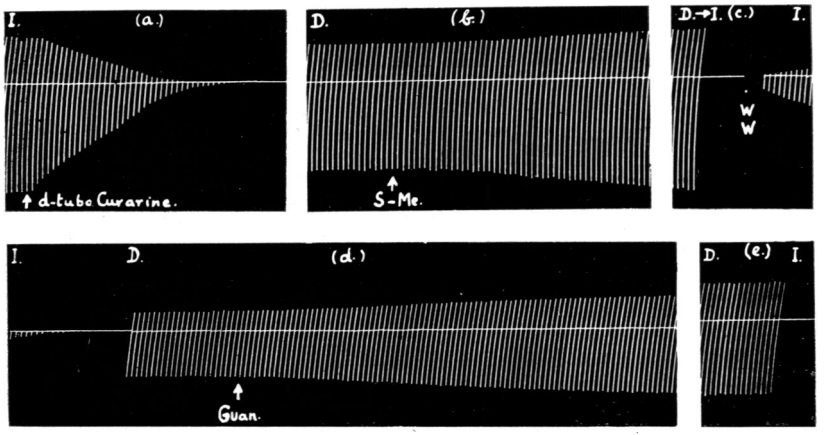

FIG. 4.—Rat diaphragm preparations. I indicates indirect and D direct stimulation. (*a*) The effect of maximal indirect shocks (1.7 m.amp., 0.5 msec. shocks at 10 sec. intervals) is abolished by a 200 μg. dose of *d*-tubocurarine. (*b*) Same preparation. Contractions now elicited by direct electrical stimulation. Methyl *iso*thiourea hydrochloride (M/4,000 at the arrow) enhances the response to these, although (*c*) indirect stimulation shows that the preparation remains fully curarized. After two washings, sensitivity to indirect stimuli slowly returns. (*d*, *e*) A similar experiment with guanidine hydrochloride (M/500 at the arrow). There is a 10 min. interval between strips (*d*) and (*e*).

Influence of calcium ion concentration. — A reduction in the effective concentration of calcium ions in the bath was brought about (i) by adding sodium oxalate solution to the bath, (ii) by adding sodium citrate solution, or (iii) by replacing the ordinary Ringer-Tyrode solution in the bath with a solution containing less than the normal amount of calcium chloride. It was noted, confirming McDowall (1949), that a moderate reduction in the calcium ion concentration of the bath usually increases the muscle twitch, whereas a large reduction invariably decreases it. In the latter instance, adding calcium chloride solution restored the amplitude of the twitch; the normal effect of an increase in calcium ion concentration was to reduce the twitch.

In six experiments guanidine (M/500) was added to Ringer-Tyrode solution containing half the normal amount of calcium. The average increase in the size of the maximal twitches in these six experiments was definitely less than that observed in seven control experiments in which the same dose of guanidine was added to normal Ringer-Tyrode solution. Raising the calcium content of the bath was less effective for counteracting the action of guanidine. One of the most striking features of this set of experiments was the persistence of the guanidine effect. Even ten or fifteen washings failed to restore the normal reaction of the preparation. Consequently little could be learned from the effect of a second dose.

Similar experiments were carried out with methyl *iso*thiourea. As a test dose, 0.5 ml. of an aqueous M/10 solution was injected into the 100 ml. bath. In ordinary Tyrode solution this dose of methyl *iso*thiourea caused a slow steady increase in the size of the twitch in almost all of some twenty trials. The response to the *iso*thiourea was reduced —sometimes quite suppressed—when the calcium ion concentration was lowered sufficiently to reduce the amplitude of the twitch; but it was not much affected by doubling or quadrupling the calcium content of the bath.

Interaction with potassium.—Raising the potassium ion concentration of the Ringer-Tyrode solution in the bath at first potentiates increas-

ingly the response to a maximal indirect shock, then reduces it—just as does a typical amidine derivative when it is given in increasing amount.

It was observed that after two 20 mg. doses of potassium chloride (at which time a further increase in the potassium ion concentration of the 100 ml. bath could no longer increase the size of the twitch), test doses of methyl or ethyl *iso*-thiourea were unable to cause potentiation. Conversely, a test dose of potassium chloride given after a large dose of a short-chain amidine derivative produced much the same sort of effect as would be expected if excess potassium were already present. In experiments with guanidine this change in the reaction of the preparation was still seen after eight or ten washings—which is in accord with the persistence of other effects of guanidine. Amongst alkyl *iso*thioureas, the *n*-hexyl derivative was found to antagonize the potentiating effect of potassium chloride more effectively than those with shorter side-chains.

Cat sciatic nerve-gastrocnemius preparation

Methyl *iso*thiourea sulphate was tested in doses of 2–5 mg./kg. on eleven cats. Almost as soon as the drug solution entered the iliac artery the "maximal" contractions produced by stimulating the muscle through the sciatic nerve six or twelve times a minute were increased, usually by some 10–20 per cent (Fig. 5*c*). When given in the same range of doses guanidine hydrochloride had practically no effect, while *n*-hexyl *iso*thiourea hydrobromide decreased the height of the contractions.

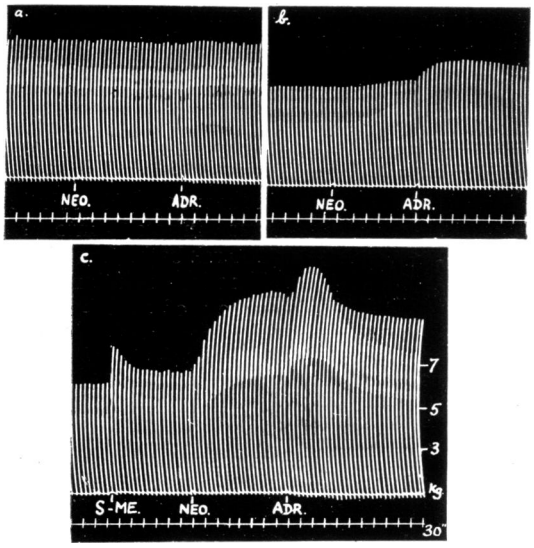

FIG. 5.—Cat ♀, 2.8 kg., under chloralose. Sciatic nerve-gastrocnemius muscle preparation. Contractions elicited by maximal shocks at 10 sec. intervals. Neostigmine given at 90 min. intervals (doses of 10 μg. at NEO) followed 2–5 min. later by adrenaline (10 μg. at ADR). Note the potentiated response to neostigmine after the administration of methyl *iso*thiourea sulphate 10 mg. at S-Me).

If muscle contractions were first decreased some 20–30 per cent by giving a small dose of *d*-tubocurarine, methyl *iso*thiourea caused more striking potentiation than usual. If, however, enough *d*-tubocurarine was given to produce an 80–90 per cent decrease, the effect of a subsequent dose of methyl *iso*thiourea was relatively slight.

In eight experiments small doses of neostigmine (3–6 μg./kg.) were given every 90 minutes. Even this long interval between doses was usually insufficient to prevent cumulation entirely. However, cumulation alone seemed inadequate to explain the greatly enhanced effect of a test dose of neostigmine given after either methyl *iso*thiourea or guanidine (Fig. 5). In three of the four experiments made with each compound it appeared certain that the amidine derivative had potentiated the effect of the neostigmine.

Adrenaline had little or no effect on this preparation when given in a dose of 3–5 mg./kg., but after the administration of neostigmine the test dose of adrenaline usually produced a considerable increase in the size of the muscle twitches. No corresponding effect was seen in parallel experiments with guanidine or methyl *iso*thiourea. However, in several preparations in which the adrenaline caused only a decrease in the size of the contractions, this depressant effect was seen to be enhanced after methyl *iso*thiourea had been given.

DISCUSSION

In their effects upon smooth muscle preparations amidine derivatives like methyl *iso*thiourea and guanidine resemble histamine rather than adrenaline. This is shown especially by the experiments with blocking agents. Whereas the vasconstrictor action of methyl *iso*thiourea on the rabbit's ear preparation was antagonized appreciably by "Anthisan" (an anti-histamine drug), it was hardly affected by "Priscol" (which is predominantly an anti-adrenaline). Not all actions of histamine on muscle are reproduced, however. The amidine derivatives tested showed no tendency to relax rat uterus. They differed from histamine moreover in acting on striped muscle almost as strongly as on smooth.

When effects on striped muscle preparations are taken into consideration it becomes evident that

these amidine derivatives resemble potassium more closely than histamine: like potassium, they potentiate the effect of "maximal" electrical shocks delivered to rat diaphragm muscle through the phrenic nerve; they also antagonize the effect of low concentrations of *d*-tubocurarine on the rat diaphragm preparation whilst having their own effects reduced by higher concentrations of *d*-tubocurarine—which suggests that they compete with it for the same receptors (cf. Quilliam and Taylor, 1947). Moreover, potassium salts in moderate excess resemble typical amidine derivatives in causing the contraction of most types of smooth muscle; they also have a vasoconstrictor action which is not affected appreciably by the treatment with ergotoxine, apart from one caused by the liberation of adrenaline (Mathison, 1911; Knoefel and Alles, 1938; Fastier and Smirk, 1947; Sugawara and Tada, 1927).

It must be added that the majority of these effects can also be reproduced by a reduction in calcium ion concentration. Thus in comparing the effects of guanidine on frog rectus muscle with those of calcium deprivation, Harvey (1940) observed that "both bring about a sensitization to potassium ions, their action is antagonized by excess of calcium, and both lead to a 'spontaneous' activity." The close correspondence between symptoms of guanidine poisoning and those of parathyroid tetany has long been known (Paton and Findlay, 1917).

Nevertheless, so closely linked are the physiological actions of potassium and calcium that Harvey and others have been content to attribute the pharmacological effects of guanidine to changes in ionic balance rather than to anything so specific as calcium lack or potassium excess. Neither possibility is ruled out by the above results, but it seems significant that the chemical relatives of methyl *iso*thiourea which have been found to resemble it at all closely in pharmacological properties are those whose salts ionize freely to yield small cations (Fastier, 1948). In order to explain the effects of these various bases in terms of "potassium excess" one need only assume that they can simulate more or less closely the action of potassium ions, unlike their higher homologues, which show a far greater tendency to produce only inhibitory effects (Fastier and Reid, 1948). It is far less easy to explain how a reduction in the effective concentration of calcium ions might be brought about.

Whatever be the precise explanation, it is obvious that amidine derivatives have a deep-seated action on muscle. A diversity of muscle-contracting agents have their effects modified in the presence of amidine derivatives. Antihistamine effects on gut and bronchioles have been observed with large doses of amidine derivatives and potentiation of histamine effects with smaller doses. Analogous effects upon sensitivity to the vasoconstrictor action of adrenaline have been described by Fastier and Reid (1948). Pharmacological effects of acetylcholine too may be modified by typical amidine derivatives.

It has been known for some considerable time that guanidine can potentiate the action of acetylcholine on striped muscle (Frank, Stern, and Nothmann, 1921; Harvey, 1940). The finding that bases like *asym*-dimethylguanidine, methyl and ethyl *iso*thiourea also possess this property is hardly surprising considering the numerous other analogies that have been noted in their pharmacological behaviour (Fastier and Smirk, 1947). That guanidine can sensitize frog rectus muscle to potassum as well as to acetylcholine has been shown by Harvey (1940). The ability of guanidine to potentiate the effects of a variety of muscle-contracting agents is further evident from results of Camis (1909), Paton and Findlay (1917), Fühner (1920), and Kato (1939) on striped muscle, and of Fühner (1917), Burns and Watson (1920), Godeaux (1942), and García and Perdomo (1946) on smooth muscle preparations. In the rat phrenic nerve-diaphragm preparation the increased response to "maximal" electrical shocks applied indirectly may well depend solely upon an enhanced sensitivity to the acetylcholine liberated at myoneural junctions. However, this leaves unexplained the observation that some potentiation can still be obtained after curarization.

Guanidine has been found to be of some value for the treatment of myasthenia gravis (Minot, Dodd, and Riven, 1939), though why it affords relief to myasthenics is far from evident. Guanidine does not inhibit cholinesterase (Minot, 1939; Thompson and Tice, 1941). Therefore it might be concluded that the action of this drug is quite different from that of neostigmine; but Thompson and Tice question such a view. They point out *inter alia* that the duration of relief of symptoms in myasthenia gravis after neostigmine follows the change in serum potassium more closely than it does the decrease in activity of cholinesterase. Like Cumings (1939), they believe that some abnormality of potassium metabolism is present in myasthenia gravis. The above results suggest that potentiation of the effect of acetylcholine is but one manifestation of a more general action of guanidine on striped muscle, and possibly it is

not this but some related effect which is of therapeutic value in myasthenia gravis. It may be remarked in conclusion that, as several other amidine derivatives have been shown to resemble guanidine closely in their effects upon striped muscle preparations, some of them might also prove of clinical value.

Summary

1. Effects of methyl *iso*thiourea and guanidine salts have been studied on perfused rabbit ears, isolated strips of rabbit and guinea-pig ileum, isolated rat uterus, guinea-pig bronchioles (Konzett-Rössler technique), isolated frog rectus muscle, cat sciatic nerve-gastrocnemius, and rat phrenic nerve-diaphragm preparations. A few experiments have also been performed with salts of other alkyl *iso*thioureas and of methyl- and *asym*-dimethylguanidine.

2. The short-chain amidine derivatives tend to increase the tonus of smooth muscle, though their effect on some preparations is negligible. The vasconstrictor response to methyl *iso*thiourea, unlike that of adrenaline, is not antagonized appreciably by "Priscol," but both the vasoconstrictor action of methyl *iso*thiourea and its tonus-increasing action on gut are partially antagonized by the anti-histamine drug "Anthisan."

3. The effects of methyl *iso*thiourea and of guanidine on striped muscle preparations resemble those of potassium in excess. They are able to potentiate the effect of maximal electrical shocks applied indirectly to the rat diaphragm preparation; they exert an appreciable anti-curare action; and their potentiating action on the rat diaphragm preparation is not readily antagonized by *d*-tubocurarine.

4. Several of the short-chain amidine derivatives have been shown to enhance the response of frog rectus muscle to test doses of acetylcholine. Potentiation of effects of histamine on gut and bronchioles has also been observed under conditions described in the text. References to yet other potentiating actions of amidine derivatives on muscle are given.

5. The mode of action of these compounds is discussed, particularly in relation to that of histamine and of potassium.

It is a pleasure to acknowledge here my indebtedness to Professor J. H. Burn, who has afforded me every facility for carrying out this work and has constantly guided its progress.

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