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CIRCULATORY PROPERTIES OF AMIDINE DERIVATIVES .II. POTENTIATION OF THE VASOCONSTRICTOR ACTION OF ADRENALINE

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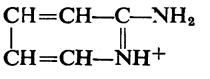
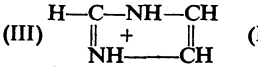
A capacity to enhance the pressor action of adrenaline in anaesthetized animals is almost as conspicuous in methyl *iso*thiourea* (I) and its nearer homologues as their own pressor activity. Both properties have been described before in some detail (McGeorge, Sherif, and Smirk, 1942 ; Fastier and Smirk, 1943), and evidence has been obtained which points to the blood vessel wall as the main site of action for pressor as well as potentiating effects.

Various other amidine derivatives of fairly low molecular weight have since been found to produce closely corresponding changes in sensitivity to the pressor and vasoconstrictor actions of adrenaline. Results obtained with derivatives of the type $X.C(:NH_2+)NH_2$—e.g., ethyl *iso*-urea, propionamidine, and *asym*-dimethylguanidine, where $X=C_2H_5O$-, C_2H_5-, and $(CH_3)_2N$- respectively—have already been reported (Fastier and Smirk, 1947). That even the presence of substituents in the amidine group itself does not invariably preclude this kind of activity will be shown below for such bases as S,N-ethylene *iso*thiourea, 2-aminopyridine (II), and iminoazole (III).

(I) $CH_3S.C(=NH_2+)NH_2$

(II) 

(III) 

(IV) $CH_3(CH_2)_8S.C(=NH_2+)NH_2$

(V) $H_2N—C(=+H_2N).S(CH_2)_6S.C(=NH_2+)—NH_2$

Nevertheless "higher" amidine derivatives as a class seem to behave quite differently. So far from enhancing sensitivity to adrenaline when injected in moderately large doses, long-chain amidine derivatives such as *n*-nonyl *iso*thiourea (IV) and hexamethylene di-*iso*thiourea (V) bring about no less emphatic *de*sensitization. Their effects upon the blood pressure, discussed in the preceding paper (Fastier, 1948), appear equally anomalous at first sight. However, a more intensive study of the activity displayed by these compounds has indicated how such contrasting observations may be reconciled ; as will also be shown below, an appreciation of the ambivalent character of typical amidine derivatives goes a long way towards explaining differences in effects upon sensitivity to adrenaline, outstanding though these may appear superficially.

* Represented conveniently as a kation for reasons given before (Fastier and Smirk, 1947).

METHODS

Experiments were performed upon dogs and cats anaesthetized with sodium barbitone and upon pithed rat hind-quarters perfused at a constant rate, as described in another paper (Fastier and Smirk, 1947). Ringer-Locke solution aerated with oxygen containing 5 per cent carbon dioxide was used for all perfusion experiments. Salts of the amidine derivatives were dissolved in Ringer-Locke solution to give the dilutions specified in the text, a little alkali being added as required when the resulting solution was acidic.

"Autosensitization"—an increasing sensitivity in the response of the preparation to adrenaline owing to intrinsic causes (*vide* Jang, 1940, and others whom he quotes)—was normally encountered at an early stage of each experiment. However, after some 30–60 min. a stage was usually reached at which the response to adrenaline had become substantially constant, provided that "cumulative" effects were also avoided by keeping the interval between successive injections to 6 or, on occasion, to as much as 9 or 12 min. Even so, the changes in sensitivity brought about by a given amidine derivative were sometimes difficult to assess because, as in the process of autosensitization, the height and the duration of the response to adrenaline were not always equally affected.

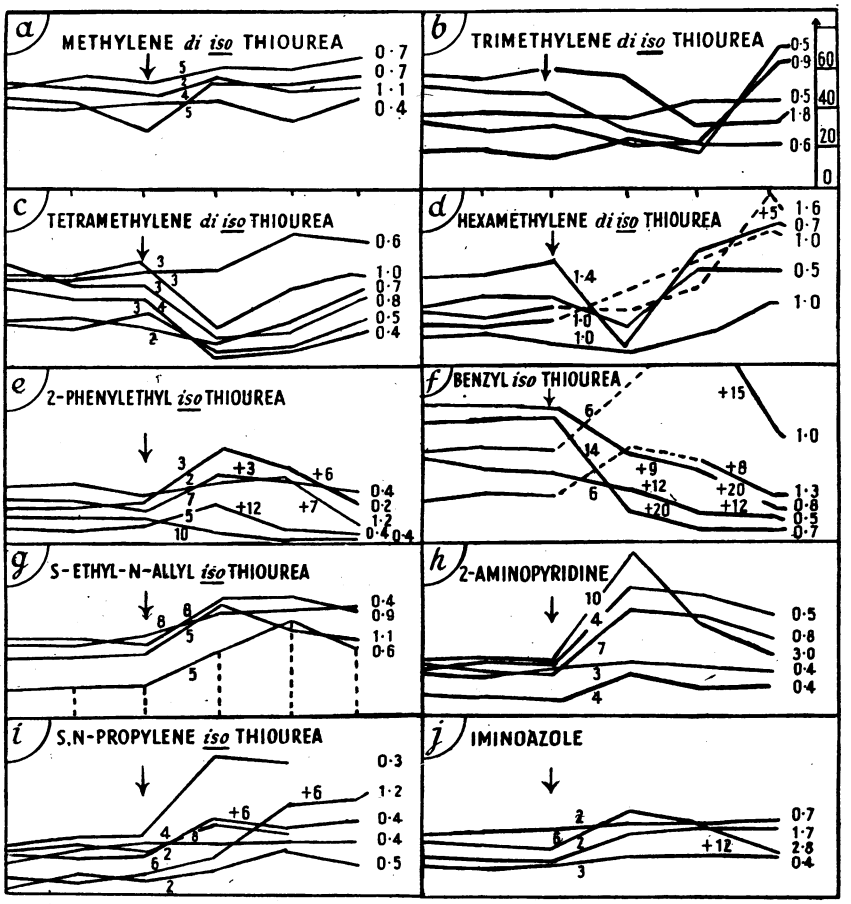


FIG. 1.—Effects of various amidine derivatives upon the sensitivity of anaesthetized dogs to the pressor action of adrenaline. Each "sensitivity" curve was constructed as shown under (g), where the dotted ordinates are the rises of blood pressure caused by the injection of small doses of adrenaline at 6-minute intervals reduced to a common base line. The rise after the heavy arrow, which marks the point of injection of the amidine derivative (dose in mg./kg. given alongside each curve), thus indicates an enhanced response to a fixed dose of adrenaline (dose given in μg./kg. to the right of each curve). Dotted curves (d,f) refer to experiments where the dose of *iso*thiourea was injected very slowly.

The pressure changes due to the amidine derivative itself provided an additional complication. Plotting the heights of the initial pressure response to adrenaline was thought to provide as convenient and reliable an index as any of sensitizing and desensitizing effects, and this has been the procedure adopted in constructing Figs. 1 and 2.

RESULTS

Experiments were first performed on anaesthetized dogs and cats. The results illustrated in Fig. 1 are typical of those obtained. They show that a variety of amidine derivatives are able to enhance the pressor action of adrenaline when their salts are injected in doses of the order of 1–10 mg./kg. They also show, however, that some amidine derivatives normally produce adrenalytic effects when tested under these conditions (Fig. 1c, d, f) and that others are far from constant in effect (Fig. 1b, e).

In order to see whether clear-cut results could be obtained with compounds of the latter type, two or three of them were given in widely graded doses. It so came to be noticed that while desensitization was produced eventually as the dosage was increased, sensitization was usually obtained at an earlier stage of the experiment. Some of the

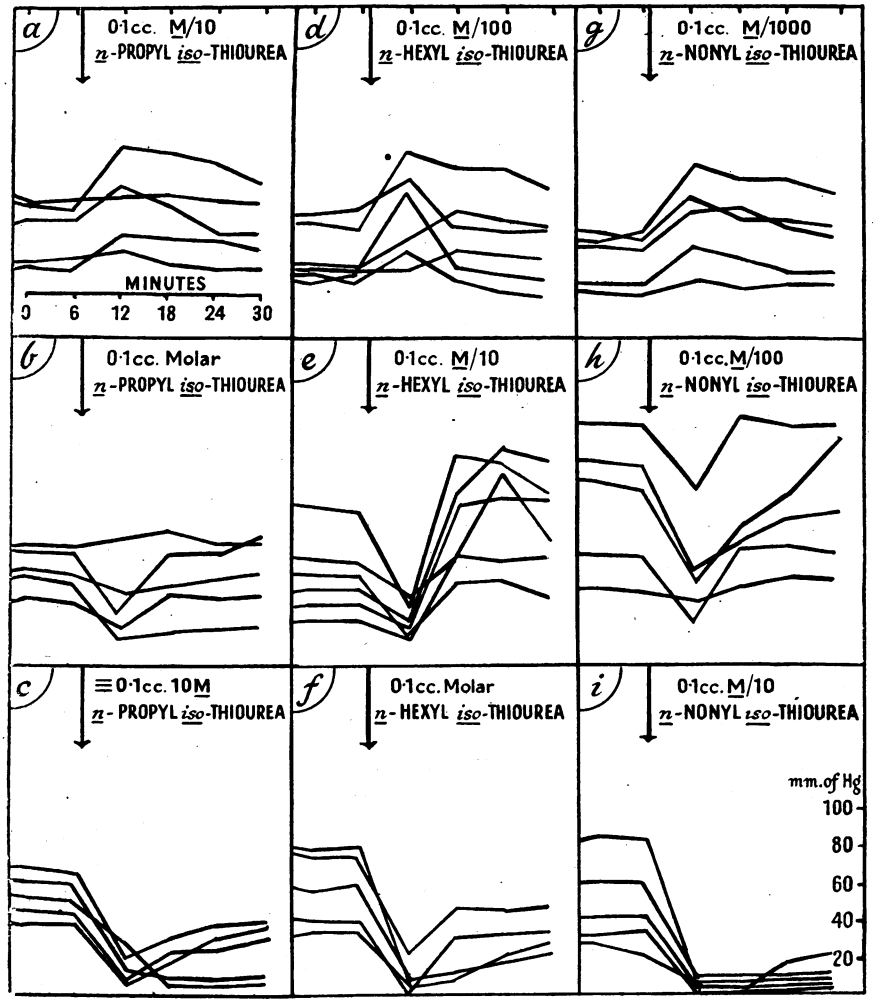


FIG. 2.—Pithed rat hind-quarters preparation. The sensitivity curves shown in this diagram were constructed as indicated under Fig. 1 but illustrate changes in the height of successive *vasoconstrictor* responses to a fixed dose of adrenaline (usually 0.1 μg.) given at 6-minute intervals. Thus in the five experiments represented in the top left-hand corner, the slope of the curves indicates strong sensitization to adrenaline after the injection of a moderate dose of *n*-propyl *iso*thiourea. The same compound can be seen to have produced equally emphatic desensitization in experiments in which larger doses were given (b,c).

Note that both sensitization and desensitization to adrenaline can be produced also by higher homologues in appropriate doses (d,e,f; g,h,i) although the effect of a given dose, say 0.1 c.c. of an *M*/10 solution (a,e,i), varies greatly from one compound to another.

"pure" adrenalytics were then examined. Even these, it was discovered, were quite capable of producing sensitization to the pressor action of adrenaline; a dose which would have caused immediate desensitization if washed into the circulation all at once brought about no less emphatic sensitization, initially at any rate, when administered over a period of 20–30 minutes (Fig. 1d, f). Consequently, the suspicion grew that the response to an amidine derivative might in general be influenced qualitatively as well as quantitatively by dosage. The following experiments were therefore carried out with a more typical compound, effects upon sensitivity to adrenaline being investigated on pithed rat

hind-quarters in preference to anaesthetized animals in order to simplify conditions as far as possible.

Changes in the sensitivity of perfused rat blood vessels to the vasoconstrictor action of adrenaline induced by various doses of n-*hexyl* iso*thiourea hydrobromide*

When small quantities (0.05–0.20 c.c.) of solutions varying in concentration from $M/100{,}000$ to $2M$ were injected one minute before the next dose of adrenaline was due, a threshold for sensitization was found with a concentration of the order of $M/10{,}000$. As the concentration of the *iso*thiourea solution was increased, the sensitization produced increased in extent, becoming maximal for a concentration of the order of $M/100$. The vasoconstrictor response to adrenaline was frequently doubled with this dose (Fig. 2d).

With somewhat larger doses, however, the initial effect was a lesser degree of sensitization; and when the dosage was increased still further, actual desensitization resulted (Fig. 2e, f). The desensitization was usually transient; in such instances (Fig. 2e) a subsequent phase of sensitization followed regularly, which seems highly significant when it is recalled that the perfusion fluid does not circulate in the rat hind-quarters preparation as blood circulates in the intact animal; fresh Ringer solution is constantly being pumped into the dorsal aorta to renew that escaping from severed vessels. If a drug is not held tenaciously by the tissues it must in consequence soon be washed out of the preparation. Presumably, when such a dose as 0.1 c.c. of the $M/10$ solution is injected, the local (i.e., the "effective") concentration of *n*-hexyl *iso*thiourea rises sufficiently high for it to exceed a threshold "desensitizing level." It would appear that this concentration cannot be maintained, however, and as it gradually diminishes, recovery takes place, to be followed by a period of sensitization. Even with strongly adrenalytic doses, which might initially reduce the height of the vasoconstrictor response to adrenaline to one quarter or even one tenth of its former value (Fig. 2f), this pattern was normally followed.

The above interpretation of the form of the sensitivity curves shown in Fig. 2d, e, f, was confirmed by experiments in which *n*-hexyl *iso*thiourea was administered by injecting it into the reservoir of Ringer-Locke solution instead of directly into the perfusion cannula. As but 15–20 c.c. of Ringer-Locke solution entered the preparation each minute, diluting the *iso*thiourea in some 200–400 c.c. of the perfusion fluid ensured that it could not rapidly achieve a high concentration in the tissues even if the amount ultimately reaching the preparation was large. Under these conditions it was found possible to demonstrate that desensitization is definitely preceded by sensitization when a large dose of *iso*thiourea is given (as would be expected if the type of effect produced depends mainly upon "effective" concentration). Moreover, desensitization occurred the more rapidly the higher the concentration of *n*-hexyl *iso*thiourea ($M/20{,}000$, $M/5{,}000$, $M/2{,}500$, $M/1{,}000$) or the faster its rate of perfusion. Recovery followed the reverse pattern. Thus on returning to plain Ringer-Locke solution after perfusing an $M/5{,}000$ solution of *n*-hexyl *iso*thiourea, normal sensitivity was regained within a few minutes and followed by fairly long-lasting sensitization. Only in those experiments in which one of the stronger solutions had been perfused for a considerable time was sensitization not observed after changing back to ordinary Ringer-Locke solution, apparently because the dose had reached a toxic level, judged by the persistent loss of irritability of the preparation.

Sensitizing and desensitizing effects of homologues of n-*hexyl* iso*thiourea*

Nine other *iso*thioureas of general formula $CH_3(CH_2)_nS.C(:NH_2^+)NH_2$ were subsequently tested on the pithed rat hind-quarters preparation, *viz.*, methyl, ethyl, *n*-propyl, *n*-butyl, *n*-amyl, *n*-heptyl, *n*-octyl, *n*-nonyl, and *n*-decyl *iso*thioureas, for which $n=0$, 1, 2, 3, 4, 6, 7, 8, and 9. Their effects upon sensitivity to adrenaline all seemed to be influenced by dosage in essentially the same manner as those of *n*-hexyl *iso*thiourea (for which $n=5$).

Lower homologues differ from the *n*-hexyl member mainly in that sensitization is obtained over a wider range of concentrations. The doses required for the demonstration of desensitization with *n*-propyl *iso*thiourea (Fig. 2c) or with methyl or ethyl *iso*thiourea are definitely "unpharmacological."

With higher homologues, on the other hand, adrenalytic activity was the predominant feature. As shown in Fig. 2i, the desensitization produced by *n*-nonyl *iso*thiourea in such a dose as 0.1 c.c. of the $M/10$ solution is intense. Nevertheless even *n*-nonyl *iso*thiourea was found capable of producing the opposite effect when given in sufficiently small amount (Fig. 2g), as were also *n*-heptyl, *n*-octyl, and *n*-decyl *iso*thioureas.

Likewise with the anaesthetized dog, in which *n*-nonyl and *n*-decyl *iso*thiourea seemed at first to be purely adrenalytic, it was found possible to demonstrate sensitization by using a very small amount for the first injection and gradually work-

ing up the dose. Still higher members ($n=11$, 13, 15) gave little indication of activity; because of their insolubility they were not tested on the rat hind-quarters preparation. The lower members of the series in doses of the order of 1–10 mg./kg. enhanced the pressor action of adrenaline in anaesthetized dogs, and this property extended well up the series (from $n=0$ to $n=7$).

The pithed rat hind-quarters technique is the better adapted, however, to showing the variation encountered during the ascent of the series. Thus with 0.1 c.c. of the $M/100$ solution as the fixed dose, a gradual increase was apparent in the degree of sensitization produced, a maximum being reached at about the heptyl derivative. At the same time the period of detectable activity increased from some 15–20 minutes to upwards of 30–40 minutes. However, from the propyl derivative upwards, there was an increasing delay before maximum sensitization was observed. The desensitization level was actually exceeded for a time with this dose of *n*-hexyl *iso*thiourea and higher homologues, although in order to prove the point for the heptyl and octyl derivatives the first dose of adrenaline had to be injected as soon as 30 seconds later; desensitization was not observed in those experiments in which the interval was increased to 2 or 3 minutes. Its transitory occurrence was demonstrated even with the hexyl derivative, however, by injecting the adrenaline in a steady stream instead of in doses given at regular intervals, and thus obtaining a continuous measure of sensitivity. No such device was necessary with the highest homologues tested; the desensitization obtained with *n*-nonyl and *n*-decyl *iso*thiourea was considerable and relatively persistent even with this small dose (Fig. 2h).

Sensitizing and desensitizing effects of other amidine derivatives

With the di-*iso*thioureas of general formula $H_2N(+H_2N:)C.S(CH_2)_n S.C.(:NH_2+)NH_2$, increasing the length of the polymethylene chain ($n=1$–6) was likewise found to enhance adrenalytic activity. It has been shown above (Fig. 1c, d) that the tetramethylene and hexamethylene derivatives produce desensitization quite consistently in anaesthetized dogs in doses as low as 2–5 mg./kg. In the rat hind-quarters preparation their adrenalytic activity was equally pronounced. Sensitization of the perfused blood vessels was also obtained with most of these di-*iso*thioureas, although it could not be demonstrated regularly with the tetramethylene and hexamethylene derivatives even when their salts were perfused in high dilution. It was an inconstant phenomenon also with benzyl and 2-phenylethyl *iso*thiourea salts in the rat hind-quarters preparation. That these particular adrenalytics were able under some conditions at least to cause sensitization as well as desensitization to adrenaline was obvious enough, however, from the results obtained with anaesthetized dogs (Fig. 1e, f) and cats.

A much closer resemblance to lower homologues, like methyl and *n*-propyl *iso*thiourea, was displayed by the N-substituted amidine derivatives S,N-ethylene and S,N-propylene *iso*thiourea, 2-aminopyridine (II), and iminoazole (III). They too produce sensitization in the rat hind-quarters preparation even when injected in fairly large amounts. For the demonstration of desensitization, doses equivalent to 0.1 c.c. of an M-10M solution were required. Much smaller doses sufficed, however, with bases like S-ethyl-N-allyl *iso*thiourea and 2-aminoquinoline which, though allied structurally to the above N-substituted amidine derivatives, have considerably larger kations.

Discussion

Nearly all the amidine derivatives whose activity has been studied in detail have been found capable, like *n*-hexyl *iso*thiourea, of producing either sensitization or desensitization to the vasoconstrictor action of adrenaline, the latter effect becoming predominant sooner or later when the dosage was increased. Differences in their effects appear to be quantitative rather than qualitative; for while the effect of a fixed dose varies greatly from one compound to another, a distinctive pattern can be discerned none the less if the *series of effects* produced by a wide range of doses is made the basis of comparison, as indicated in Fig. 2.

Perhaps the strongest evidence in favour of this idea that both "lower" and "higher" amidine derivatives produce essentially similar sensitizing and desensitizing effects lies in the gradual transition from one characteristic type of behaviour to the other seen during the partial ascent of the two homologous series of *iso*thioureas tested. One plausible objection may be raised, however. Why does the effect produced by a fixed dose alter so considerably during the ascent of a homologous series if all the homologues produce a given effect by the same mechanism?

As Ferguson (1939) has properly emphasized in connexion with toxicity data, we must distinguish between "chemical" and "physical" attributes in comparing the pharmacological activity of two drugs. The fact that one of the "higher" members of an *iso*thiourea series is able to produce the same pharmacological effect (e.g., desensitiza-

tion to the vasoconstrictor action of adrenaline) as one of its lower homologues when it is given in a much smaller dose does not necessarily mean that 1 molecule of the higher homologue is as effective as, say, 10 or 100 molecules of the lower homologue *at the site of action*; it might well be that the distribution co-efficient of the lower homologue was relatively so unfavourable that 10 or possibly 100 times as many molecules of *iso*thiourea had to be present in the enveloping medium (the "external phase") in order to maintain the same number of molecules as the higher homologue at the site of action (the "biophase").

To press the argument a little further, we might interpret the above results as follows: both "lower" and "higher" amidine derivatives are able to influence the sensitivity of blood vessels to adrenaline in a characteristic manner by virtue of a particular *chemical* configuration (the charged amidine "head" of the molecule) which enables them to react with the same receptors; but they differ quantitatively in their effects owing to variation in the *physical* properties of the molecule as a whole, which leads in turn to variation in their capacity to reach the appropriate receptors or remain in contact with them. Evidence believed to favour this possibility will be presented in a later paper.

Summary

1. It has been shown for the first ten *iso*thioureas ($n=0$–9) of general formula:

$$CH_3(CH_2)_nS.C(:NH)NH_2$$

that either sensitization or desensitization to the vasoconstrictor action of adrenaline in the pithed rat hind-quarters preparation may be observed after their administration, according to the experimental conditions employed.

2. Which effect is produced seems to depend mainly upon dosage. Sensitization to a varying extent will occur so long as the local concentration of the *iso*thiourea falls within certain limits (which are characteristic of a given compound); but once the hypothetical upper limit is exceeded, desensitization appears and becomes increasingly emphatic if the local concentration of the base rises still higher.

3. Experiments with various other strongly basic amidine derivatives—e.g., methylene di-*iso*thiourea, hexamethylene di-*iso*thiourea, S,N-propylene *iso*thiourea, benzyl *iso*thiourea, 2-aminopyridine, iminoazole—suggest that they too show a qualitative resemblance to the alkyl *iso*thioureas in producing first sensitization and then desensitization to the vasoconstrictor action of adrenaline when they are given in increasing concentration.

4. Adrenalytic effects are observed more especially with the higher members of a series—e.g., *n*-octyl *iso*thiourea, tetramethylene di-*iso*thiourea, and their near homologues. The desensitization to the pressor action of adrenaline which they produce in anaesthetized dogs probably depends (like the sensitization obtained with smaller doses) in part if not entirely upon some process occurring in the blood vessels themselves, since desensitization to the vasoconstrictor action of adrenaline can be readily demonstrated with these "higher" amidine derivatives in the pithed rat hind-quarters preparation.

5. The influence of chemical structure upon this kind of activity is discussed.

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