Brit. J. Pharmacol. (1960), **15**, 365.

THE RESPONSES OF THE *VENUS* HEART TO CATECHOL AMINES AND HIGH CONCENTRATIONS OF 5-HYDROXYTRYPTAMINE

BY

M. J. GREENBERG*

From the Biological Laboratories, Harvard University, Cambridge, Massachusetts, U.S.A.

(RECEIVED JANUARY 28, 1960)

The catechol amines excite the isolated heart of *Venus mercenaria* in a characteristic manner. This response was not obtained with phenethylamine, tyramine, ephedrine, or mescaline, nor with histamine, nor with the basic n-alkylamines. 5-Hydroxytryptamine had a distinctive effect at high concentrations (above 3×10^{-6} M) different from that at lower doses. The response to high concentrations was dominated by an increase in muscle tone. Hearts exposed to high concentrations of 5-hydroxytryptamine and other tryptamine analogues for long periods became tachyphylactic to low doses of these substances. However, high doses of 5-hydroxytryptamine (about 2×10^{-5} M) still excited the tachyphylactic heart, but the response was then like that to the catechol amines. When high bath temperatures rendered the heart insensitive to 5-hydroxytryptamine, high concentrations of this compound again had the catechol amine effect. The possibility of a physiological role for the catechol amines or high 5-hydroxytryptamine concentrations is discussed.

While studying structure-activity relations on the heart of *Venus mercenaria* (Greenberg, 1960), attention was drawn to the effects of some catechol amines and high concentrations of 5-hydroxytryptamine.

The responses of lamellibranch hearts to adrenaline and noradrenaline are varied (Krijgsman and Divaris, 1955; Welsh, 1953; Fänge, 1955; Gaddum and Paasonen, 1955). For example, most hearts are excited by adrenaline ; when treated with high concentrations they are arrested in systole. On the other hand, the oyster (Jullien, 1936) and *Anodonta cygnea* (Fänge, 1955) show negative inotropic effects when treated with adrenaline. Again, *Amblema peruviana* (Motley, 1934) and *Cardium edule* (Gaddum and Paasonen (1955) are arrested in diastole by high concentrations of catechol amines.

Only a few workers have tested both catechol amines and 5-hydroxytryptamine on the lamellibranch heart (Welsh, 1953 ; Fänge, 1955 ; Gaddum and Paasonen, 1955). In the species tested, the hearts have always been found to be more sensitive to 5-hydroxytryptamine than to adrenaline or noradrenaline. In only three species was there a notable qualitative difference between the two excitatory actions.

High concentrations of 5-hydroxytryptamine have never been studied, probably for the good reason that the physiological role of this substance as excitatory neurohumour (Welsh, 1957) is likely to be associated with near-threshold concentrations.

Tachyphylaxis to 5-hydroxytryptamine was demonstrated by Gaddum on guinea-pig ileum in 1953. It has since been observed in a number of mammalian preparations (Gaddum and Hameed, 1954), but has never been produced in molluscan preparations.

In the present study on the *Venus* heart the mode of excitation by catechol amines is examined over the entire range of action. High concentrations of 5-hydroxytryptamine are also tested and it is found that they can induce tachyphylaxis to moderate concentrations of this substance. When such desensitization has been established, however, high 5-hydroxytryptamine doses evoke responses similar to those of the catechol amines. The structure-activity relations of this response are briefly studied. The possibility that there is a physiological role for the catechol amines in *Venus* is suggested.

*Present address: Department of Zoology, University of Illinois, Urbana, Illinois, U.S.A.

METHODS

Preparation.—Large specimens of *Venus mercenaria* (clams; quahogs), obtained fresh from Narragansett Bay, were employed. The hearts were removed in the manner described by Welsh and Taub (1948). The excised ventricles were immersed in a jacketed, aerated 10 ml. bath and stretched between a stainless steel hook and an isotonic lever balanced either at 500 or 1,000 mg. The perfusion fluid in the bath proper was filtered sea water obtained from Woods Hole, Mass. Water from a reservoir, thermostatically maintained at 15°, was circulated through the bath jackets. Responses were recorded on a kymograph with a smoked drum.

Drugs were made up in standard solutions of 10^{-1} M to 10^{-3} M. These were serially diluted with distilled or sea water. The dose in a volume of 0.1 to 1.0 ml. was added directly to the bath fluid with a hypodermic syringe. Proper mixing was provided by the stream of aerating bubbles. The addition of 1.0 ml. of distilled water alone has no effect on the clam heart. Most doses are expressed as moles per litre, but concentrations of Brom LSD and Mytolon in the bath are given as g./ml.

The length of exposure of a heart to a particular dose varied between a min. and over an hr. depending upon the compound and the concentration used. At least 5 min. were always allowed between washing and the addition of the succeeding dose.

Benzoquinonium chloride (Mytolon; 2:5-bis (3′ diethylaminopropylamino)-benzoquinone bis benzyl chloride) (10^{-5} g./ml.) was maintained in the bath except during the washing process. This drug is an effective antagonist of acetylcholine in the *Venus* heart (Luduena and Brown, 1952). In these experiments benzoquinonium was used to improve the regularity of the beat by preventing the depression of the heart probably caused by endogenous acetylcholine. Benzoquinonium has no effect on the response of the heart to 5-hydroxytryptamine.

The Effect of High Bath Temperature on the 5-Hydroxytryptamine Effect.—By increasing the temperature of the water in the reservoir, that of the circulating water in the jackets could be raised to 30° or 35°. Temperature equilibrium between the jacket water and the sea water in the bath occurred in about 5 min. Ten min. were always allowed.

The Effect of pH Change on the Performance of the Isolated Venus Heart Preparation.—Aliquots of sea water were adjusted with hydrochloric acid or sodium hydroxide so that the *p*H varied from 4.2 to 9.0. In testing the effects of *p*H on the heart, normal sea water in the bath was rapidly withdrawn and replaced by the altered sea water. The hearts were equilibrated at the new hydrogen ion concentration for 15 min.

Specificity.—Brom LSD (2-brom-(+)-lysergic acid diethylamide) (10^{-5} g./ml.) is an antagonist of 5-hydroxytryptamine on the *Venus* heart (Welsh and McCoy, 1957). This antagonism is believed to be specific. In the present study Brom LSD is used to test the specificity, relative to 5-hydroxytryptamine, of the various exciter agents employed.

Drugs Used.—The following compounds were used in the course of this study: Tryptamine hydrochloride, 3,4-dihydroxyphenylethylamine (dopamine) hydrochloride (Mann Research Laboratories); 5-hydroxytryptamine creatinine sulphate (Nutritional Biochemicals); 2-brom-(+)-lysergic acid diethylamide (Sandoz, Inc.); phenethylamine, heptylamine, *n*-hexylamine, *n*-amylamine, *n*-butylamine, ethylamine (Eastman Kodak); tyramine (Abbott Laboratories); (−)-epinephrine bitartrate, levarterenol bitartrate monohydrate, benzoquinonium chloride (Sterling-Winthrop); ephedrine hydrochloride, mescaline hydrochloride (Hoffman-LaRoche); histamine diphosphate (General Biochemicals); bufotenine, 5-hydroxy-α-methyltryptamine, N′N′-dimethyltryptamine (Upjohn).

RESULTS

The Catechol Amine Effect.—The effects of adrenaline, noradrenaline and dopamine on the *Venus* heart were similar, but differed from those of 5-hydroxytryptamine.

The catechol amines produced a series of responses which varied qualitatively with concentration. There were two sorts of effects in the range of action. At the low-concentration end there occurred a decrease in amplitude with no chronotropic effect. Since these experiments were done in the presence of benzoquinonium this response could not have been due to a stimulation of the acetylcholine receptors of the heart. At higher concentrations an increase in tone, concomitant with an increase in frequency, predominated. At the extreme high end of the concentration range, the response was immediate systolic arrest.

The effects of adrenaline and noradrenaline were almost identical (Fig. 1). The pure negative inotropic effect was a relatively small part of the response occurring just at threshold doses (10^{-5} to 5×10^{-5} M) (Fig. 1a and c). A slight increase of the threshold dose by $1–2 \times 10^{-5}$ M resulted in a response which was a mixture of the negative inotropic and positive chronotropic effects as well as an increase in tone ($2–7 \times 10^{-5}$ M). A dose of adrenaline or noradrenaline 5 to 10 times larger than that which just increases the tone caused systolic arrest ($7–12 \times 10^{-5}$ M) (Fig. 1a).

The action of dopamine differed from that of adrenaline and noradrenaline in two respects. Firstly, the potency of dopamine, with regard to the increase of tone and frequency, was about 10 times that of adrenaline or noradrenaline (Fig. 1c). Secondly, the decrease in amplitude played a more prominent part in the response to dopamine. There was a good deal of variation, but a negative inotropic effect was always obtainable at 2 to 3×10^{-6} M (Fig. 1c). A decrease in amplitude of more than 50% was not unusual. Increases of tone

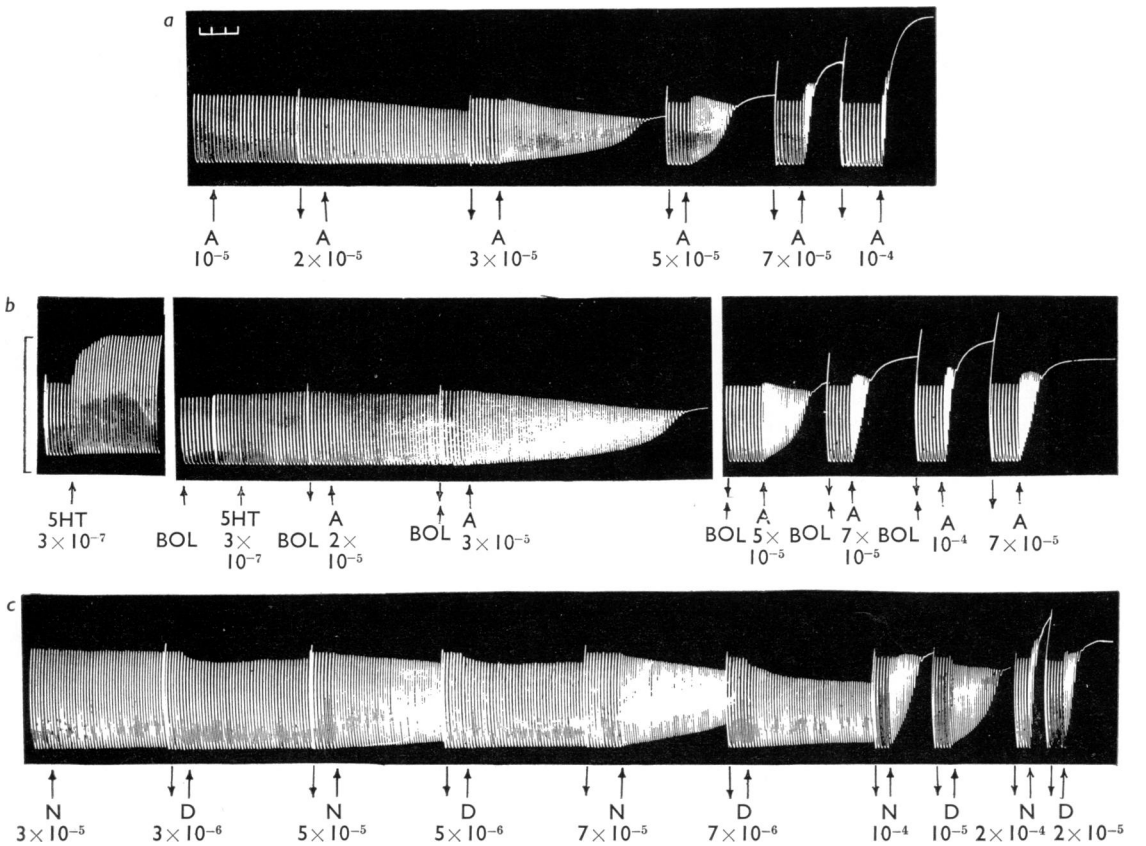

FIG. 1.—The response to catechol amines of the isolated *Venus* heart preparation. (*a*) The effect of adrenaline A. (*b*) Same preparation. The effect of adrenaline (A) in the presence of 2-bromo-(+)-lysergic acid diethylamide (BOL) (10^{-6} g./ml.). First dose of BOL added 20 min. before excitator agent (5-hydroxytryptamine (5HT)); maintenance doses immediately after washing and 5 min. before each dose of exciter. (*c*) Comparison of noradrenaline (N) and dopamine (D) on the same preparation. Benzoquinonium chloride (10^{-5} g./ml.) added to bath 5 min. before each dose. Downward-pointing arrows indicate washing. Dose: moles/litre. Tension: 1,000 mg. Temperature: 15°. Time scale: 30 sec. Amplitude scale: 5 cm.

were usually produced by a dose of 5 to 20 times greater than threshold.

The responses to catechol amines were not blocked by Brom LSD (10^{-5} g./ml.) (Fig. 1b); this distinguishes these responses from those to 5-hydroxytryptamine.

It is important to know whether the catechol amine effect is merely an unspecific response of the heart to high concentrations of drug or whether it results from an action at specific sites in the tissue. A partial answer to this question can be obtained by examining the responses of the heart to various analogues of the catechol amines.

Responses to Other Phenethylamine Analogues.—Tyramine and phenethylamine were tested at concentrations between 10^{-5} M and 10^{-4} M. The response of the heart was 5-hydroxytryptamine-like, although 500 to 4,000 times weaker. The positive inotropic effect was antagonized by Brom LSD. This surprising result is dealt with elsewhere (Greenberg, 1960).

The actions of ephedrine and mescaline were relatively feeble even at such high concentrations as 10^{-4} M to 10^{-3} M. The effects of these compounds were qualitatively similar to those of tyramine and phenethylamine. Mescaline had about one third, ephedrine one fiftieth the potency of phenethylamine. No attempt was made to block the actions of these two compounds with Brom LSD.

None of the above analogues of phenethylamine tested produced an effect resembling that of the catechol amines at any concentration.

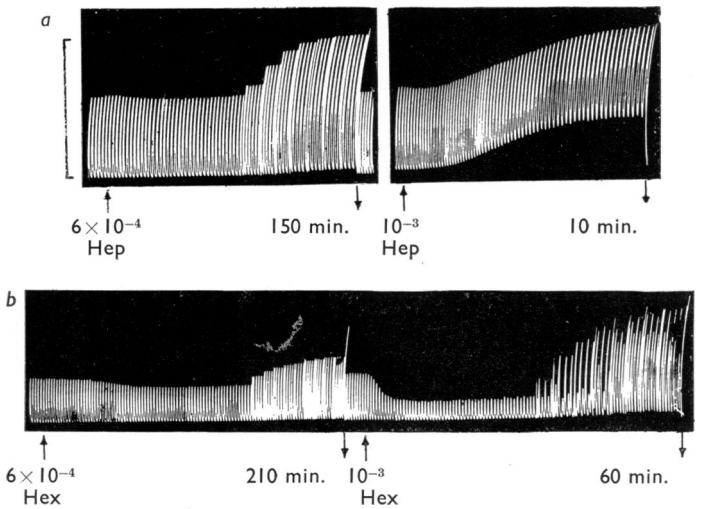

FIG. 2.—The response of the isolated *Venus* heart preparation to *n*-alkylamines. (*a*) Heptylamine (Hep). (*b*) Hexylamine (Hex). Total time for each response is indicated in min. Downward-pointing arrows indicate washing. Benzoquinonium chloride (10^{-5} g./ml.) was added to the sea water 5 min. before each dose. Dose: moles/litre [illegible] 00 mg. Temperature: 15°. Time scale: 30 sec. Amplitude scale: 5 cm.

Responses to Histamine (*4-Ethylamino Imidazole*).—Histamine is a well-known smooth muscle exciter. It acts occasionally on lamellibranch hearts in relatively high concentrations (Pilgrim, 1954; Waddum and Paasonen, 1955). Histamine has a curious effect on the *Venus* heart. Threshold concentration, when there was any action at all, was about 10^{-6} M. The response was a rapid increase in amplitude of beat which fell off again fairly rapidly. The increase in amplitude was equivalent to that produced by about 4×10^{-9} M 5-hydroxytryptamine. Increasing doses of histamine, up to 10^{-4} M, did not increase the effect; in fact, the response sometimes became smaller.

The action of histamine was not blocked by Brom LSD and hence histamine was not acting on the 5-hydroxytryptamine site. No sign of a catechol-amine-like effect has been observed in response to histamine.

Responses to n-*Alkylamines*.—The *n*-alkylamines tested excited the *Venus* heart in high concentrations. However, the mode of excitation was unique: neither like that of the catechol amines, nor histamine, nor 5-hydroxytryptamine.

Threshold, for amyl-, hexyl-, and heptylamine, was between 10^{-4} and 3×10^{-4} M. The effect was a transient decrease in amplitude lasting about 10 min. and followed by an increase in amplitude which took about an hour to develop (Fig. 2). Both the positive and negative inotropic effects increased only slightly with increasing doses up to 8×10^{-4} M. At 10^{-3} M the amines had a sudden violent effect the nature of which depended upon the compound used. With heptylamine there was a great increase of tone and a slowing of rate (Fig. 2). Hexylamine and amylamine produced an augmentation of the transient decrease and then a great irregularity of beat (Fig. 2). As might be expected the alkylamine effect was not blocked by Brom LSD (10^{-5} g./ml.).

The *n*-alkylamines with a chain length of less than five carbons had a smaller potency. Ethylamine and butylamine had about one third of the activity of hexylamine; ammonia had one sixth to one tenth of the activity of hexylamine.

The perfusion fluid, sea water, is buffered naturally. In the course of storage, prior to use, the *p*H dropped to about 7.8. The addition to the [illegible] of relatively large concentrations of these basic amines caused the *p*H to increase to as much as 8.4. Four heart preparations were tested to determine their tolerance to changes of hydrogen ion concentration. The normal functioning of the hearts was independent of *p*H. The frequency was stable at about 10 beats per min. between *p*H 5.9 and 8.6. The amplitude, while stable between *p*H 4.8 and 9.0, dropped noticeably at 4.2. Thus, between *p*H 7.8 and 8.4, the range employed in the study of the *n*-alkylamines, there was no *p*H-induced variation in the normal amplitude and frequency of the hearts.

The Effect of 5-Hydroxytryptamine.—The response of the *Venus* heart to 5-hydroxytryptamine has three components: an increase in amplitude, an increase in frequency, and an increase in the resting tone of the muscle. The relative importance of these components varies with the concentration (see Figs. 3 and 4).

Between threshold concentration (about 10^{-9} M) and moderately high doses (about 10^{-6} M) the response of the *Venus* heart to 5-hydroxytryptamine was mainly an increase in the amplitude of beat. There was also an increase in tone, which increases with concentration, and an increase in frequency which, however, was not dependable. A plot of the final amplitude of the response (measured from the original baseline) against log concentration is a

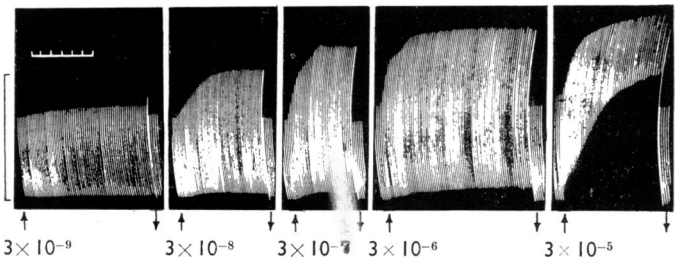

FIG. 3.—The effect of 5-hydroxytryptamine (5HT) on the isolated *Venus* heart preparation. Downward-pointing arrows indicate washing. Benzoquinonium chloride (10^{-5} g./ml.) added to sea water 5 min. before each dose of 5HT. Dose: moles/litre. Tension: 1,000 mg. Temperature: 15°. Time scale: 30 sec. Amplitude scale: 5 cm.

sigmoid curve which levels out at about 10^{-6} M and then rapidly increases with higher concentrations.

Above 10^{-6} M 5-hydroxytryptamine the relative increase of the tone became larger. At 10^{-5} M the effect was almost all increase in tone while the rhythmical excursions of the heart were very small. The ventricle was often arrested in systole. When the increase in tone is plotted as a function of log concentration a really large increase in the slope starts rather suddenly at about 3×10^{-6} M (Fig. 4). At these high concentrations the frequency of beat often doubled or trebled.

Thus, there appears to be two distinct responses of the *Venus* heart to 5-hydroxytryptamine. One, at low to moderate concentrations, is dominated by a positive inotropic effect. Tone is relatively unimportant and frequency changes are not dependable. The other response, evoked by high concentrations, is dominated by a large increase in muscle tone. The inotropic effect no longer followed the relationship implied by the sigmoid curve found at lower concentrations. The frequency always increases noticeably.

A measure of the 5-hydroxytryptamine effect at lower concentrations is the difference, in mm., between the amplitude of beat before and after the addition of a dose (b–a in the inset of Fig. 4). It is interesting that, from threshold to about 10^{-6} M 5-hydroxytryptamine, the plot of this effect against log concentration was the expected sigmoid curve. Above 10^{-6} M, when the tone also suddenly increased greatly, the points ceased to follow this relationship (Fig. 4). The action of 5-hydroxytryptamine in concentrations below 10^{-6} M is dealt with elsewhere (Greenberg, 1960). The present work concerns the effects of doses greater than 3×10^{-6} M.

Brom LSD (10^{-5} g./ml.), given 20 min. previously, antagonized the action of moderately high doses of 5-hydroxytryptamine ($3–10\times10^{-7}$ M). This blockade was usually surmountable by concentrations of

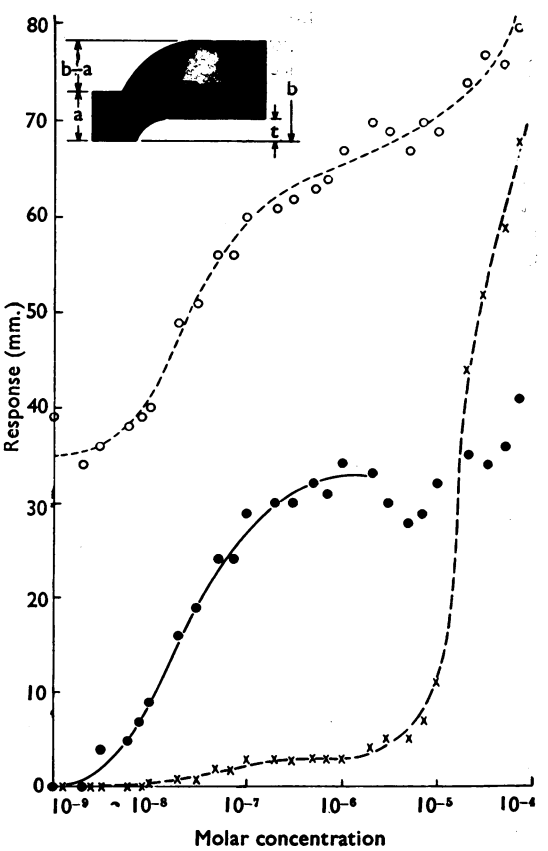

FIG. 4.—The effects of increasing concentrations of 5-hydroxytryptamine on the performance of the isolated *Venus* heart preparation (see Fig. 4). (X) indicates the amount of tone (t) in the response. (O) indicates the final amplitude (b) of the response. (●) represents the difference (b–a) between the final (b) and initial (a) amplitudes. (t), (b), (a), and (b–a) are explained in the inset, which is a diagram of a 5-hydroxytryptamine response.

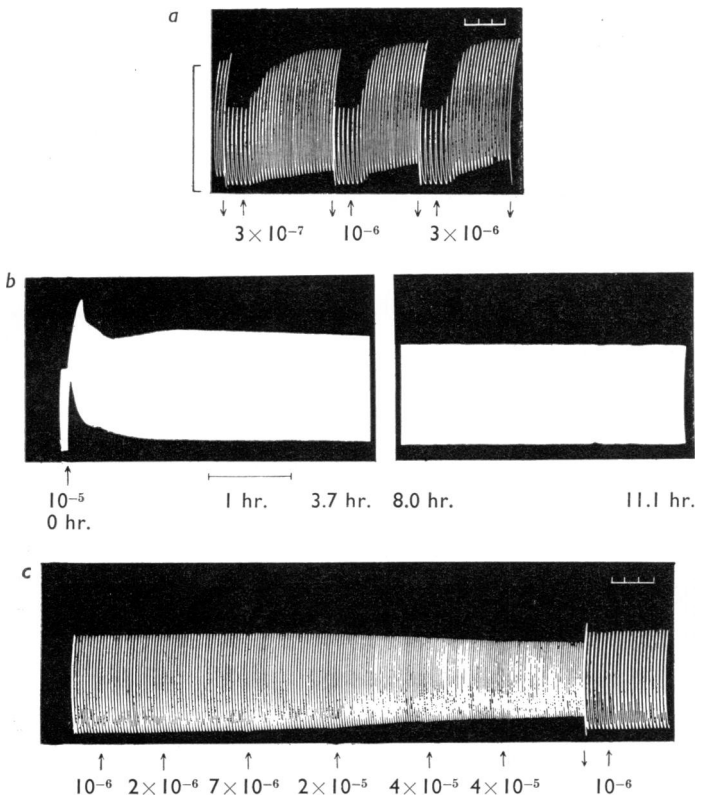

FIG. 5.—Tachyphylaxis of a *Venus* heart preparation to 5-hydroxytryptamine. (*a*) Three standard doses of 5-hydroxytryptamine (5HT) successively washed out (indicated by down-pointed arrow). (*b*) Record made at slow drum speed (Time scale : 1 hr.) showing the secondary decrease in amplitude following a large dose of 5HT. (*c*) Large doses of 5HT now added to tachyphylactic preparation. (*a*), (*b*), and (*c*) are consecutive records from the same preparation. Benzoquinonium chloride (10^{-5} g./ml.) added after every washing. Dosage: moles/litre. Tension: 500 mg. Temperature: 15°. Time scale for (*a*) and (*c*) : 30 sec. Amplitude scale: 5 cm.

5-hydroxytryptamine greater than 10^{-6} M or by smaller doses if the preparation was a sensitive one. Often, the *Venus* heart was excited by 10^{-5} g./ml. Brom LSD (see Greenberg, 1960). Increased time of application or increased dose resulted in substantial excitation. Consequently, Brom LSD is not useful as an inhibitor of high 5-hydroxytryptamine doses.

Tachyphylaxis to 5-Hydroxytryptamine.—When 10^{-5} M 5-hydroxytryptamine was present in the bath the tone of the heart increased greatly. If the dose was not washed out, this effect slowly diminished. After from 4 to 12 hr. the ventricle was beating almost normally (Fig. 5b). The tone and amplitude may be larger than before the application of the large dose of 5-hydroxytryptamine, but usually not as large as it is in response to even moderate concentrations (10^{-8} to 10^{-7} M). Such a preparation was then tachyphylactic. It did not respond to the further addition of increasing doses of 5-hydroxytryptamine up to 10^{-5} M (Fig. 5c). It also did not respond to 5-hydroxytryptamine analogues, such as tryptamine and bufotenine (Fig. 6a). Furthermore, hearts could be made tachyphylactic to tryptamine (Fig. 8a), bufotenine, or 5-hydroxy-α-methyltryptamine (Fig. 6b), and afterwards these hearts will be unresponsive to 5-hydroxytryptamine as well as to the desensitizing drugs. On the other hand, the catechol amines still excited desensitized hearts in their characteristic manner, although the magnitude of the response may be reduced (Fig. 6c).

Isolated *Venus* hearts which have been left untreated for 12 hr. responded to *all* 5-hydroxytryptamine concentrations in the same manner as fresh preparations. Thus, tachyphylaxis does not depend upon the age of the preparation. It was not possible to restore the sensitivity of the tachyphylactic heart to 5-hydroxytryptamine even after prolonged washing. Specific tachyphylaxis to 5-hydroxytryptamine has been described in guinea-pig ileum and other mammalian tissues (Gaddum, 1953; Gaddum and Hameed, 1954). Tachyphylaxis, in these tissues, occurs relatively rapidly and is reversible.

High 5-Hydroxytryptamine Concentrations after Tachyphylaxis.—Desensitized hearts were insensitive to doses of 5-hydroxytryptamine up to 10^{-5} M. However, when challenged with 2×10^{-5} M, the beat of such a heart *decreased* in amplitude. With increasing doses, up to 10^{-4} M (which caused the heart to be arrested immediately in systole), responses were produced which were almost identical with those to adrenaline and noradrenaline (Fig. 7). These catechol amine-like effects were also elicited by 5-hydroxytryptamine if the heart had been made tachyphylactic by one of its analogues (Fig. 8*a*). Furthermore, the decrease in amplitude at about

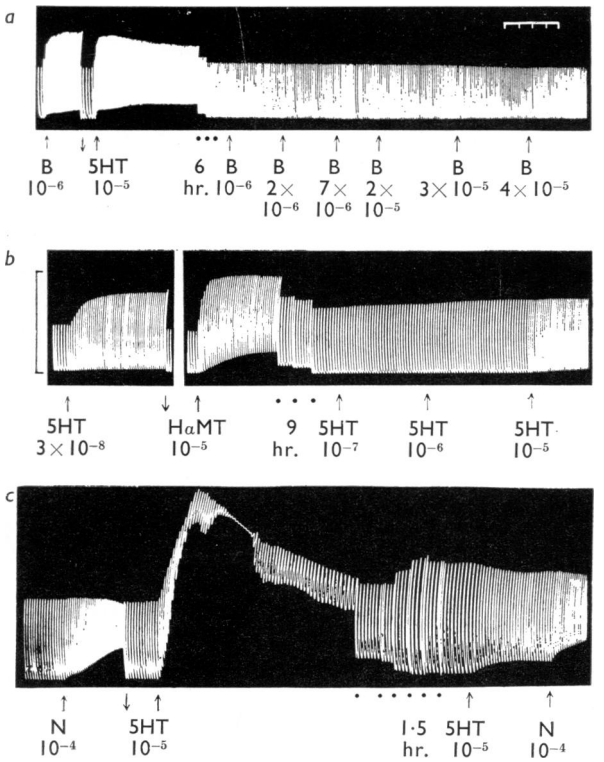

FIG. 6.—Responses of the tachyphylactic *Venus* heart preparation. (*a*) After a standard dose of bufotenine (B), the heart is made tachyphylactic to 5-hydroxytryptamine (5HT). Bufotenine, subsequently, has no effect. (*b*) After a standard dose of 5HT the heart is made tachyphylactic to 5-hydroxy-α-methyltryptamine (HαMT). Subsequent doses of 5HT are ineffective. (*c*) Tachyphylaxis produced by 5HT. The noradrenaline (N) response is relatively unaffected. (●) indicates stopping of drum. The total time required for tachyphylaxis is indicated. Washing was at the downward-pointing arrows. Benzoquinonium chloride (10^{-5} g./ml.) was added to the sea water after each washing. Doses: moles/litre. Tension: 500 mg. Temperature: 15°. Time scale: 30 sec. Amplitude scale: 4 cm.

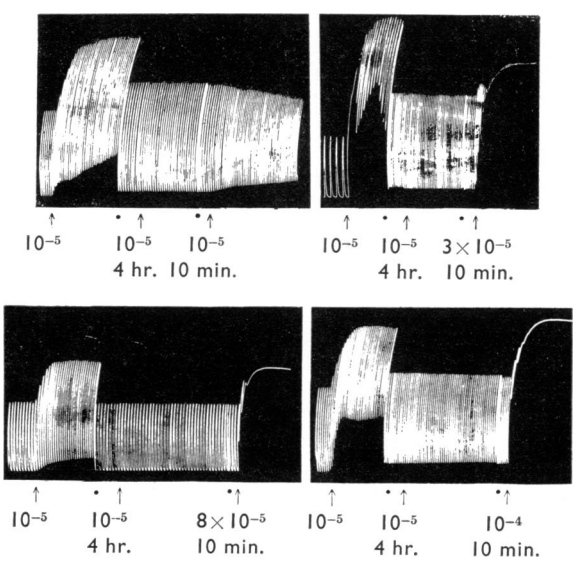

FIG. 7.—Catechol-amine-like responses (see Fig. 1) produced by 5-hydroxytryptamine (5HT) on tachyphylactic *Venus* heart preparations. (●) indicates that the drums were stopped for the time shown. Doses: moles/litre. Tension: 500 mg. Temperature: 15°.

2×10^{-5} M occurred even if the positive inotropic effect, due to a high dose of a tryptamine analogue had not disappeared (Fig. 8b). Such a decrease in amplitude after 10^{-5} M 5-hydroxytryptamine occurred when an exciting dose of Brom LSD (10^{-5} mg./ml.) was left in the bath for 7 hr. (Fig. 8c). The similarity of responses and concentrations suggests that the 5-hydroxytryptamine is acting at the same sites as the catechol amines.

It has been impossible to obtain this distinctive series of responses with either tryptamine or bufotenine. Tryptamine, at 10^{-3} M, after tachyphylaxis had, in a few experiments, produced effects resembling the *n*-alkylamine effect. Bufotenine, at least up to 4×10^{-5} M, produced only a slight decrease in amplitude with a small increase in tone on a tachyphylactic heart.

The Effect of 5-Hydroxytryptamine at High Temperatures.—High bath temperatures reduced the response of the heart to 5-hydroxytryptamine. At 30° to 35° the muscle lengthened and the addition of doses of 5-hydroxytryptamine, up to 10^{-7} M, had very little effect. When 10^{-5} M 5-hydroxytryptamine was then added there followed the decrease in amplitude which had been associated both with 5-hydroxytryptamine after tachyphylaxis and with threshold doses of catechol amines. When the bath is cooled to 15° the usual 5-hydroxytryptamine effect, at 10^{-5} M, with its high tone and small rhythmical excursions, is unmasked. Thus, it was possible to demonstrate the catechol-amine-like response to high concentrations of 5-hydroxytryptamine without desensitizing the heart to 5-hydroxytryptamine.

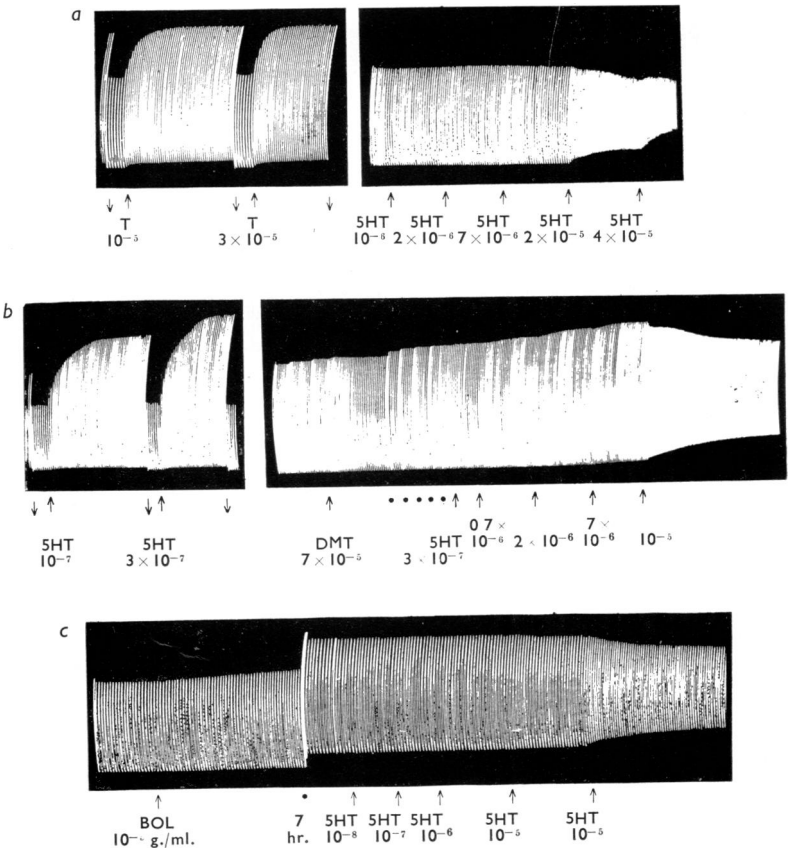

FIG. 8.—Conditions resulting in catechol-amine-like responses of 5-hydroxytryptamine. (*a*) Response of a *Venus* heart preparation to tryptamine (T) before tachyphylaxis and to 5-hydroxytryptamine (5HT) afterwards. Tachyphylaxis produced by 10^{-4} M tryptamine over a period of 11.3 hr. (*b*) Following the normal response of a heart to 5-hydroxytryptamine (5HT) are 8 hr. of application of increasing doses of *N'N'*-dimethyltryptamine (DMT), the last of which is shown. Successive doses of 5HT are then added. Note the small positive inotropic effects, up to 2×10^{-5} M 5HT, indicating the absence of tachyphylaxis. (●) indicates that drum was stopped for 7 min. (*c*) Catechol-amine-like effect of 5HT after prolonged treatment with 2-brom-(+)-lysergic acid diethylamide (BOL). Doses: moles/litre. Tension: 1,000 mg. Temperature: 15°.

DISCUSSION

A *Venus* heart preparation may be either excited or depressed by the catechol amines, depending upon the dose. Although previous workers have reported diverse effects for adrenaline and noradrenaline on lamellibranch hearts (Krijgsman and Divaris, 1955 ; Fänge, 1955 ; Gaddum and Paasonen, 1955), only for *Anodonta cygnea* is there a suggestion of more than one mode of action for

these compounds. Fänge (1955) mentions not only a negative inotropic effect, but also immediate systolic arrest at a ten-fold higher concentration. Similarly, Ten Cate (1923) and Hendrickx (1945) noted an enfeeblement of *Anodonta* heart beat prior to systolic arrest.

It seems possible that the hearts of some animals, such as *Amblema peruviana* (Motley, 1934), *Cardium edule* (Gaddum and Paasonen, 1955), and the oyster (Jullien, 1936), for which a depression by catechol amines has been reported, are especially sensitive to the negative inotropic effect of these compounds. Presumably, higher doses would evoke the excitation which has been seen in *Venus* and others. One would also expect to produce, with lower concentrations than have been used in the past, a decrease in amplitude from clam heart preparations which have been noted to be stimulated by the catechol amines.

The exciter effect of adrenaline and noradrenaline on the *Venus* heart preparation was not only of lower potency than that of 5-hydroxytryptamine below 10^{-6} M but also qualitatively different. Welsh made this distinction between the two excitatory actions in 1953.

While other lamellibranch hearts are, in general, between 1,000 and 10,000 times more sensitive to 5-hydroxytryptamine than to adrenaline, only *Cardium edule* (Gaddum and Paasonen, 1955) and *Anodonta cygnea* (Fänge, 1955) show any qualitative distinction between the two exciter effects.

The difference, on *Venus* heart, between the actions of catechol amine and 5-hydroxytryptamine suggests that these actions originate from different sites in the tissue. This contention is supported by two sorts of evidence. First, when a preparation is insensitive to low or moderate doses of 5-hydroxytryptamine, due to tachyphylaxis or high bath temperatures (30° to 35°), it will display the various effects associated with the catechol amines if challenged either by high doses of these amines or by 5-hydroxytryptamine. Second, Brom LSD (10^{-5} g./ml.), which inhibits 5-hydroxytryptamine, has little effect on the response to adrenaline. Brom LSD also produces tachyphylaxis; this has been observed only when it was exciting the heart. Therefore, it is impossible to say whether the catechol-amine-like effect would also follow an inhibition of 5-hydroxytryptamine when not preceded by excitation. However, Brom LSD does not antagonize the effect of 5-hydroxytryptamine seen in the desensitized heart; this strengthens the presumption that this effect does not occur as a result of excitation at the usual 5-hydroxytryptamine site.

It would be interesting to know whether there is in *Venus*, a physiological basis for the response to catechol amines or whether it is merely an unspecific effect of toxicological significance only. There is some evidence which suggests that a physiological role exists.

As far as has been studied, there seem to be relatively exacting structural requirements for molecules producing this effect. Thus, in contrast to 5-hydroxytryptamine, tryptamine is inactive. Phenylethylamine analogues which either lack hydroxyl groups or have methoxy groups (mescaline) in any two of the three hydroxyl positions in noradrenaline are similarly ineffective. The activity of dopamine, which has no side chain hydroxyl, suggests that only the two phenolic groups of noradrenaline are necessary for the production of the characteristic response. Whether the hydroxyl on the side chain would suffice, instead of that in the 3-position of the nucleus, must be established by testing *p*-hydroxyphenylethanolamine (octopamine). This prospect is interesting since octopamine is a natural product found abundantly in the posterior salivary glands of *Octopus vulgaris* (Bacq, Fischer and Ghiretti, 1952). The *n*-alkylamines and histamine, while excitatory, each have actions which are distinctive. The structure-activity relations of the response to the catechol amine have not been worked out. However, present information indicates that a structurally specific receptor in the cell is involved.

The responses of the tachyphylactic or overwarmed *Venus* heart to 5-hydroxytryptamine are duplicated seasonally in nature. During the summer months (late June to August) the threshold of the ventricle to 5-hydroxytryptamine may increase by ten-fold or more. In fact, normally beating hearts sometimes cannot be excited until concentrations of 10^{-6} M are reached, and then the response is small. Furthermore, in the summer, a *decrease* in amplitude of beat in response to low 5-hydroxytryptamine doses (about 10^{-8}) has occasionally been observed (B. M. Twarog ; M. K. Paasonen ; personal communications). The decrease is not acetycholine-like (see Welsh and Taub, 1948) ; the rate of beat increases and the response resembles, in fact, the effect of noradrenaline between 10^{-5} and 2×10^{-5} M. The production of the catechol amine effect by the normal excitatory neurohumour, 5-hydroxytryptamine, under naturally induced circumstances thus occurs. This suggests that the series of responses evoked experimentally by the catechol amines are physiological. No explanation for the increase of threshold of the 5-hydroxytryptamine effect in the summer is at hand. The possibility of a

relationship between summer and high temperature insensitivity and the irreversible tachyphylaxis with its slow onset is obvious and intriguing, but it has not been explored.

Finally, catechol amines have been found in the ganglia of *Venus mercenaria* (Welsh, personal communication). This strongly suggests that the catechol amines, themselves, have a role in the normal functioning of *Venus* and, more specifically, in the seasonal variation seen in the performance of the heart.

A practical result of the present work is that, as a consequence of the response of the heart to high 5-hydroxytryptamine concentrations, two tests for the specificity of action of excitatory compounds become available. The first involves the assumption that when the heart preparation is tachyphylactic to 5-hydroxytryptamine the receptors are blocked and there cannot subsequently be a response to any compound which initiates its actions by attachment to this receptor. Conversely, it is to be expected, and has been shown, that long applications of high concentrations of specifically acting compounds will also result in insensitivity to 5-hydroxytryptamine. Gaddum (1953) first used tachyphylaxis to 5-hydroxytryptamine in this way to demonstrate the existence of specific tryptamine receptors in the guinea-pig ileum.

The application of 2×10^{-5} M 5-hydroxytryptamine to the tachyphylactic *Venus* heart preparation results in a *decrease* in amplitude ; a ten-fold higher dose causes systolic arrest. This phenomenon constitutes the second means of distinguishing 5-hydroxytryptamine-like substances from other excitatory agents. It is especially useful in studies of test compounds which act irreversibly and cause tachyphylaxis, with a slow onset, after high doses.

It is a privilege to acknowledge the advice and encouragement offered by Professor J. H. Welsh during the course of this investigation.

This work was supported, in part, by a fellowship (HF-7571) from the National Heart Institute of the United States Public Health Service and by a University of Illinois Faculty Fellowship.

The author is indebted to the following for their generous gifts of chemicals: Dr. R. V. Heinzelman and Dr. M. E. Speeter, Upjohn Co. (bufotenine, 5-hydroxy-α-methyltryptamine, N'N'-dimethyltryptamine); Dr. R. Bircher, Sandoz Pharmaceuticals (2-brom-d-lysergic acid diethylamide); Dr. A. M. Lands, Sterling-Winthrop Research Institute (benzoquinonium chloride); Dr. R. K. Richards, Abbott Laboratories (tyramine).

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