Brit. J. Pharmacol. (1962), **18**, 440–450.

ACTIONS OF DERIVATIVES OF LYSERGIC ACID ON THE HEART OF VENUS MERCENARIA

BY

ANNE McCOY WRIGHT, MERILYN MOORHEAD AND J. H. WELSH

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

(*Received February 5*, 1962)

5-Hydroxytryptamine and a number of (+)-lysergic acid derivatives have been tested on the heart of *Venus mercenaria*. One group of derivatives was found to increase the amplitude and frequency of heart beat in a manner much like 5-hydroxytryptamine. It included the monoethylamide, diethylamide, propanolamide (ergometrine), butanolamide (methylergometrine) and certain peptide derivatives of lysergic acid without substituents in positions 1 or 2. Of these, lysergic acid diethylamide was the most active. Given sufficient time (up to 4 hr), as little as 10 ml. of 10^{-16} M lysergic acid diethylamide produced a maximum increase in amplitude and frequency in about one-half of the 80 hearts on which it was tested. Its action was very slowly reversed by washing, as was true of all lysergic acid derivatives. A second group of lysergic acid derivatives, substituted in positions 1 or 2, had weak excitor action, if any, and specific 5-hydroxytryptamine blocking action. This group consisted of 1-methyl-, 1-acetyl-, and 2-bromo-lysergic acid diethylamide and 1-methyllysergic acid butanolamide (methysergide). Of these, the last showed least signs of excitor action, usually none up to 10^{-4} M, and it blocked 5-hydroxytryptamine in a molar ratio of about one to one.

During the early studies on the pharmacology of the heart of the mollusc, *Venus mercenaria*, certain of the ergot alkaloids were observed to have a remarkable excitatory action that was only very slowly reversed by washing (Welsh & Taub, 1948). Later, enteramine was found to excite certain molluscan hearts (Erspamer & Ghiretti, 1951). Following the identification of enteramine as 5-hydroxytryptamine (5-HT, serotonin), this compound was shown to have a similar excitatory action on the heart of *Octopus vulgaris* (Bacq, Fischer & Ghiretti, 1952).

After blocking the released acetylcholine with benzoquinonium, electrical stimulation of the mixed regulatory nerves to the heart of *Venus* produced excitation resembling that seen after application of 5-hydroxytryptamine to the heart (Welsh, 1953, 1957). This effect of 5-hydroxytryptamine, and its presence in large amounts in molluscan nervous systems (Welsh, 1954, 1957 ; Welsh & Moorhead, 1959, 1960), provide evidence for a cardioregulatory role of this indoleamine in the molluscs. This evidence is strengthened by the many observations that have now been made of the generally similar actions of 5-hydroxytryptamine and of lysergic acid diethylamide on molluscan hearts (Welsh, 1953, 1954 ; Welsh & McCoy, 1957 ; Gaddum & Paasonen, 1955 ; Shaw & Woolley, 1956 ; Kerkut & Laverack, 1960 ; Greenberg, 1960).

Over a period of several years we have compared the actions of a number of (+)-lysergic acid derivatives, both natural and synthetic, on the isolated heart of *Venus mercenaria*. Some have been found to mimic closely the actions of 5-hydroxytryptamine and to be effective in very low concentrations, while others are effective inhibitors of this indoleamine. These results will be presented in this paper.

METHODS

Hearts of large specimens of *Venus mercenaria* were isolated and perfused with sea-water in a 10 ml. bath according to the procedure of Welsh & Taub (1948). The temperature of the bath was maintained at 15° C.

Drug solutions were made in distilled water and appropriate dilutions were added to the bath in either 0.1 ml. or 1.0 ml. volumes. Generally, a clean dry pipette was used for each addition.

The extraordinarily slow washing out of most lysergic acid derivatives required a separate heart for each test. This, and some seasonal variation in the sensitivity of the hearts, made quantitative comparisons difficult and time-consuming. For example, 10^{-16} M lysergic acid diethylamide was tested on 80 hearts at different months of the year and over a period of several years.

Lysergic acid derivatives, at low concentrations, have a slowly accumulating action on the *Venus* heart. Some were observed for periods of several hours after addition to the bath. Those having a specific blocking action were sometimes left on the heart for 4 to 5 hr before 5-hydroxytryptamine was added. Many activity comparisons were made 10 min after the addition to the bath.

Certain other procedures are given under Results and in legends to the figures.

The following drugs were used: 5-hydroxytryptamine creatinine sulphate (Nutritional Biochemicals and Regis Chemical Co).; (+)-lysergic acid; (+)-lysergic acid ethylamide; (+)-lysergic acid diethylamide (LSD); 1-methyl-(+)-lysergic acid diethylamide; 1 acetyl-(+)-lysergic acid diethylamide; ergometrine ((+)-lysergic acid propanolamide; ergonovine); methylergometrine ((+)-lysergic acid butanolamide; Methergine); methysergide (1-methyl-(+)-lysergic acid butanolamide; UML 491); 2-bromo-(+)-lysergic acid diethylamide (BOL 148); and dihydroergotamine (Sandoz Pharmaceuticals); ergotoxine (Burroughs Wellcome).

RESULTS

Because of the highly irreversible action of most of the lysergic acid derivatives, and their gradual accumulation in the *Venus* heart, it was difficult to make exact comparisons of their relative activities. The results obtained with the most active 5-hydroxytryptamine-like representative (lysergic acid diethylamide) and the most effective blocking agent (methysergide) will first be given. The results with other compounds will then be briefly mentioned in their apparent relative order of activity.

Lysergic acid diethylamide

In earlier experiments, using isolated hearts of the molluscs, *Cyprina islandica* and *Buccinum undatum*, this substance was found to have little excitor action and appeared to be an effective antagonist of 5-hydroxytryptamine (Welsh, 1956). In later studies on certain other molluscs, especially *Venus mercenaria*, it was observed to have an excitor action and an apparent 5-hydroxytryptamine blocking action was seen only when the heart was maximally excited by the lysergic acid diethylamide (Gaddum & Paasonen, 1955; Shaw & Woolley, 1956; Welsh & McCoy, 1956; Welsh, 1957; Greenberg, 1960).

Now, after several years of observation, it appears that lysergic acid diethylamide is the most effective known excitor agent for the *Venus* heart. Although there occurs some seasonal and other variation of sensitivity, over half of 80 hearts bathed with 10^{-16} M lysergic acid diethylamide have shown a maximal increase in amplitude of beat when exposed for a sufficient time (1 to 4 hr) in a 10 ml. bath. A record of such a response is shown in Fig. 1a. After this heart had been exposed to 10^{-16} M lysergic acid diethylamide for 1 hr 45 min, the addition of 10^{-8} M to the bath resulted in only a slight additional increase of amplitude after it had been allowed to act for 30 min. With some hearts, a response was seen after prolonged exposure to 10^{-17} M (Fig. 1b). On bathing the heart with 10^{-18} M lysergic acid diethylamide, the amplitude of beat gradually decreased over a period of 30 min.

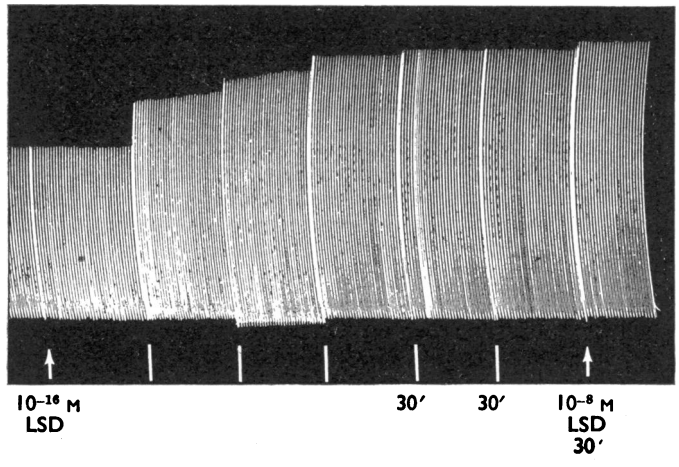

Fig. 1a. The action of 10^{-16} M lysergic acid diethylamide (LSD), followed by 10^{-8} M, on the isolated *Venus* heart. In this and following figures, at each vertical bar the drum was stopped for 10 min unless otherwise indicated. Periods of recording equal 2 min.

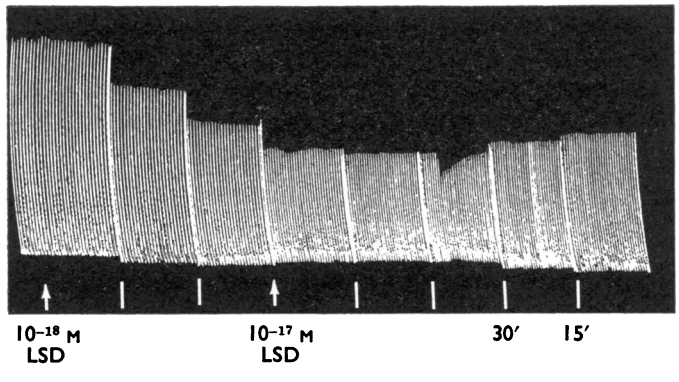

Fig. 1b. Failure of 10^{-18} M lysergic acid diethylamide (LSD) to produce an amplitude increase; followed by 10^{-17} M (LSD) which had a near threshold effect.

Such a decrease is frequently observed when fresh hearts are first bathed with sea-water. On changing the bath fluid to 10^{-17} M lysergic acid diethylamide a gradual increase in amplitude took place after an exposure of about 1 hr.

High concentrations of lysergic acid diethylamide usually act quickly. For example, 10^{-6} M usually produces a maximal increase in amplitude in less than 10 min. When an appropriate concentration of lysergic acid diethylamide is chosen, the pattern of action resembles closely that of 5-hydroxytryptamine. For example, 10^{-8} M amide may mimic very closely the time-course of the inotropic and chronotropic actions of 10^{-7} M 5-hydroxytryptamine (Fig. 2). However, one striking

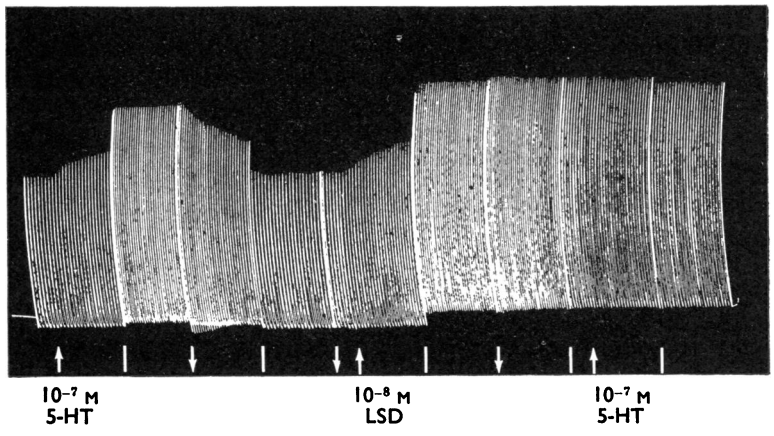

Fig. 2. Comparison of 5-hydroxytryptamine (5-HT) and lysergic acid diethylamide (LSD). At each arrow (↓) the bath was flushed three times with sea-water.

difference is apparent. The action of the latter is readily reversed on washing, while that of the former is not. After a heart is bathed for 10 to 20 min with a concentration of lysergic acid diethylamide in the range of 10^{-9} to 10^{-10} M and the heart is then repeatedly washed with sea-water, the amplitude of beat may continue to increase over a considerable period of time (Fig. 3). During 15 hr, the

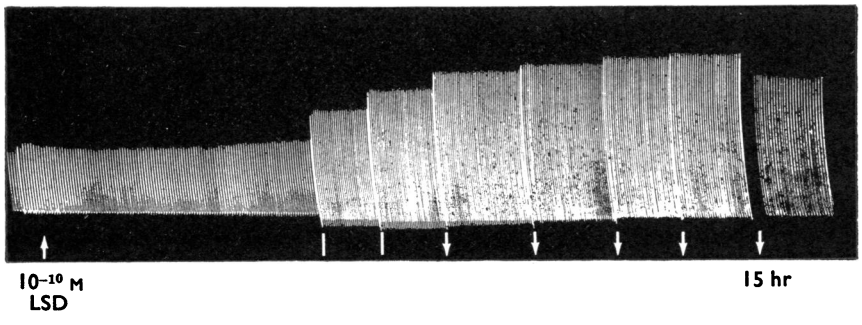

Fig. 3. At ↑, 10^{-10} M lysergic acid diethylamide (LSD). At each of four ↓, drum was stopped and bath flushed with sea-water for 10 min. At fifth ↓, sea-water was slowly run through bath for 15 hr.

heart, from which the record of Fig. 3 was taken, was washed with a slowly flowing stream of sea-water. At the end of this period the amplitude of beat was nearly twice that of the amplitude seen before the application of the drug. By increasing the *p*H of sea-water to 9.0 or 9.5 with sodium hydroxide, some of the natural ergot alkaloids (e.g., ergotoxine) may be washed out of the heart; at least the amplitude of beat returns to the level seen before the addition of the alkaloid and a second dose is effective. Attempts to reverse the action of lysergic acid diethylamide by bathing the heart with sea-water adjusted to *p*H values up to 10 were unsuccessful. Since lysergic acid diethylamide is converted to a substance which does not produce excitation by traces of free chlorine, we tried reversing its action by bathing maximally excited hearts with small amounts of sodium hypochlorite in sea-water. Within a narrow range of concentrations, near 0.001%, sodium hypochlorite would often restore to normal the amplitude of beat. But a heart so treated and then washed with many changes of sea-water failed to respond to further additions of lysergic acid diethylamide. It is possible that the oxidized lysergic acid diethylamide remains attached to receptor sites, thus preventing the unoxidized drug from acting.

Methysergide

Of the several natural ergot alkaloids and partial synthetic derivatives of lysergic acid that we have tested on the *Venus* heart, this substance is the least like 5-hydroxytryptamine in action and its most effective antagonist. At the highest concentration used (10^{-4} M), methysergide was found to have little or no effect on amplitude of beat. At lower concentrations it failed to alter the heart beat in any respect. Its effectiveness as an antagonist of 5-hydroxytryptamine is shown in Fig. 4. After application of methysergide to this heart, a washing period of 3 hr failed to restore its original sensitivity to 10^{-6} M 5-hydroxytryptamine.

Average concentration-action curves for 5-hydroxytryptamine for untreated hearts and for hearts with methysergide present in the bath are shown in Fig. 5. From these curves it is seen that the blocking ratio is approximately one to one.

Methysergide is also an effective antagonist of lysergic acid diethylamide. When 10^{-5} M of the former is allowed to remain in the bath for 30 min the addition of 10^{-6} M lysergic acid diethylamide is without obvious action over a period up to 30 min.

Other lysergic acid derivatives

As already mentioned, the other lysergic acid derivatives that have been tested on the *Venus* heart have actions that are intermediate between those of lysergic acid diethylamide and methysergide. The order of their relative actions has been only approximated due to seasonal variation in sensitivity of the heart and the difficulty of comparing one with another on the same heart. Furthermore, at equimolar concentrations, some act quickly and others slowly to produce an equivalent increase in amplitude of the beat.

The following list, with a brief summary of observations on each compound, follows the apparent order from the one most closely resembling lysergic acid diethylamide to that most like methysergide.

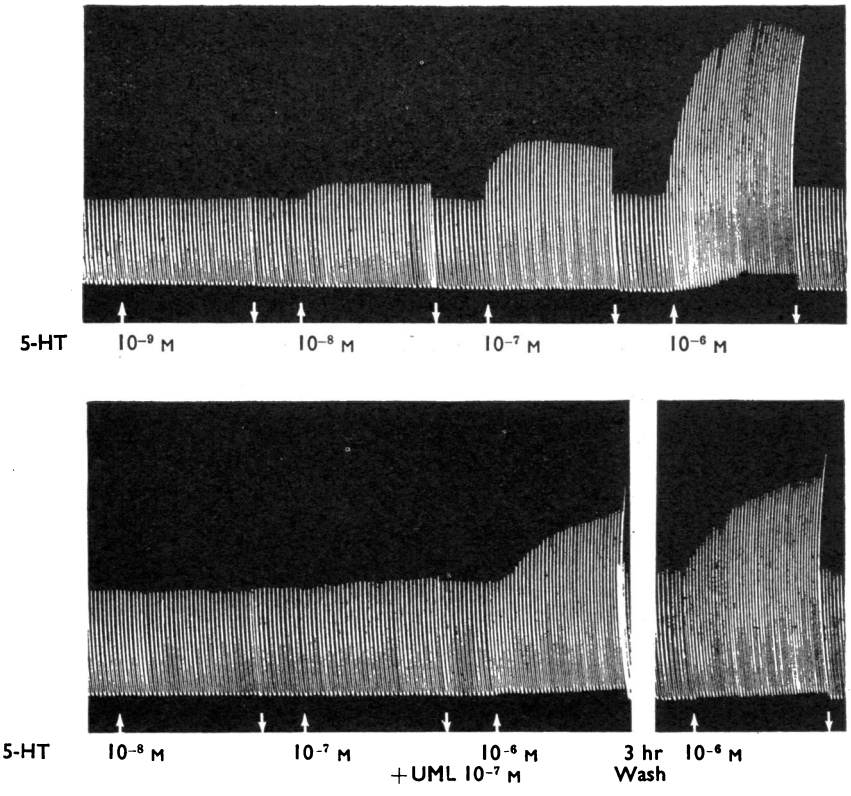

Fig. 4. Upper record shows actions of 5-hydroxytryptamine (5-HT) on *Venus* heart. In lower record, after each wash period, 10^{-7} M methysergide (UML) was added to the bath before starting kymograph. At each ↓, drum stopped and bath flushed with sea-water for 5 min. At break in lower record, heart washed for 3 hr. No methysergide in bath at second application of 10^{-6} M 5-hydroxytryptamine.

(*a*) Lysergic acid ethylamide: Strong excitor action. Threshold of winter hearts near 10^{-10} M if observed in less than 10 min after application. Slowly reversible with prolonged washing.

(*b*) Dihydroergotamine: Strong excitor action. Threshold near 10^{-10} M observed 10 min after application. Not reversed after several hours of washing.

(*c*) Ergotoxine: Strong excitor action. Threshold near 10^{-10} M.

(*d*) Ergometrine: Excitor action. Threshold near 10^{-10} M. Washed out slowly.

(*e*) Methylergometrine: Excitor action. Threshold near 10^{-8} M. Not reversed after several hours of washing.

(*f*) Lysergic acid: Excitor action. Threshold near 10^{-8} M with marked excitor action at 10^{-6} M. Action readily reversed by washing.

(*g*) 1-Methyllysergic acid diethylamide: Weak excitor action. Threshold near 10^{-7} M at 10 min. A concentration of 10^{-6} M, which does not produce maximal

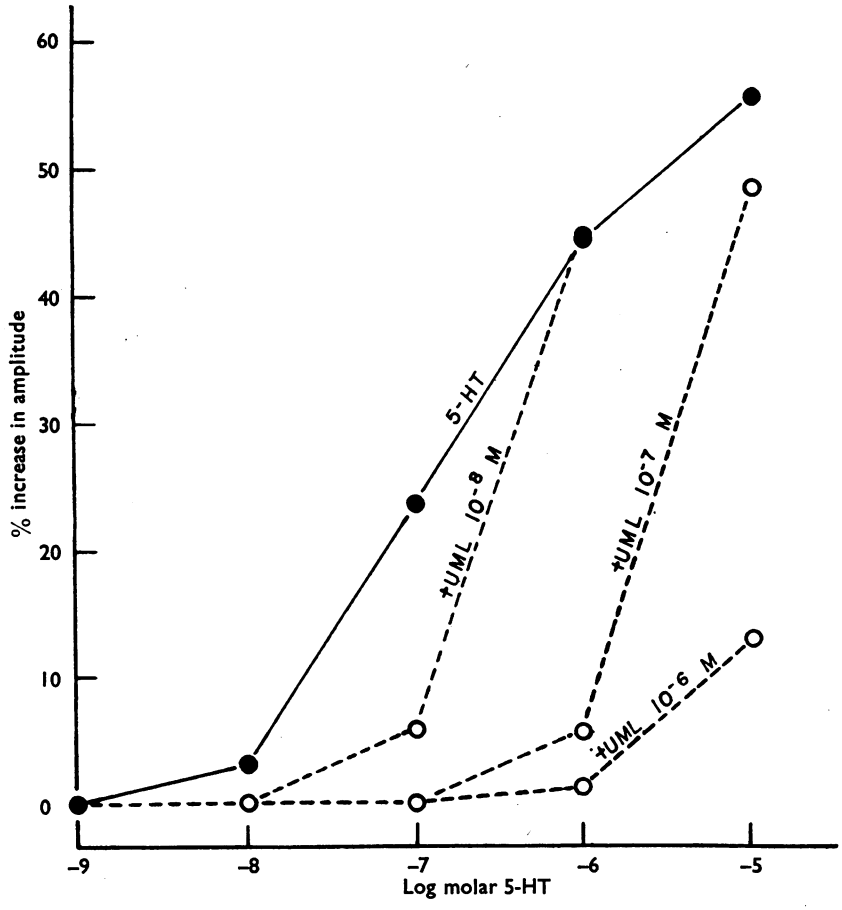

Fig. 5. Concentration-response curves for 5-hydroxytryptamine alone, and in the presence of the indicated concentrations of methysergide (UML). Each curve is the average response of three hearts.

increase in amplitude, completely antagonizes 10^{-8} M and 10^{-7} M 5-hydroxytryptamine and reduces amplitude increase produced by 10^{-6} M of the amine by 50 to 75%. Very slowly reversed with washing.

(*h*) 1-Acetyllysergic acid diethylamide: Weak excitor action. Threshold near 10^{-6} M at 10 min. In a concentration of 10^{-6} M the amide antagonizes 10^{-6} M 5-hydroxytryptamine nearly completely. Very slowly reversed with washing.

(*i*) 2-Bromolysergic acid diethylamide: Weak excitor action on some hearts at 10^{-6} M and higher; on other hearts, no excitation up to 10^{-4} M. Blocks amplitude increase produced by 5-hydroxytryptamine in approximately a one-to-one molar ratio, but tonus increase may not be fully blocked. Very slowly removed with washing. Fig. 6 illustrates its effective 5-hydroxytryptamine blocking action.

A heart treated with 2-bromolysergic acid diethylamide is not as readily excited by a subsequent application of lysergic acid diethylamide as an untreated heart, but the 2-bromo-derivative is a less effective antagonist than is methysergide.

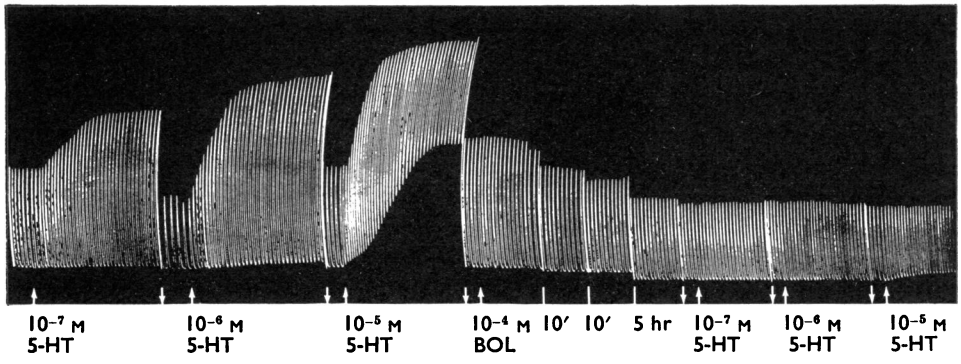

Fig. 6. Action of 5-hydroxytryptamine (5-HT) before and after 10^{-4} M 2-bromolysergic acid diethylamide (BOL). After BOL had been in bath for 5 hr 30 min, the bath was flushed and the doses of 5-HT were repeated without further addition of the amide.

DISCUSSION

The lysergic acid derivatives that have been tested on the isolated heart of *Venus mercenaria* exhibit a wide spectrum of action. Lysergic acid diethylamide has an excitor effect resembling closely that of 5-hydroxytryptamine, while methysergide fails to excite, even at high concentrations, and inhibits the response to 5-hydroxytryptamine in a molar ratio of one to one. Other derivatives are intermediate in their actions.

Concerning lysergic acid diethylamide, the following may be said: (1) it acts in very low concentrations; (2) it probably accumulates at the surface of the heart muscle cells; (3) it is bound in a highly irreversible manner; and (4) it is destroyed slowly, if at all, by the heart. Its most remarkable property is to excite the heart to beat with a maximum frequency and amplitude for extended periods of time (24 to 48 hr), with little or no sign of tachyphylaxis. 5-Hydroxytryptamine is probably the normal mediator of the cardioexcitor nerves to the *Venus* heart (Welsh, 1953, 1957; Loveland, 1962), while lysergic acid diethylamide acts as a stable slowly reversible analogue. That the physicochemical processes regulated by this more complex molecule are the same as those normally controlled by 5-hydroxytryptamine seems most likely, but it is difficult to visualize what these processes may be, to exhibit little or no signs of fatigue or adaptation for such long periods.

If evenly distributed throughout the heart, a maximal excitor action of lysergic acid diethylamide is produced by very few molecules per muscle cell. The *Venus* heart is made up of approximately 100,000 smooth muscle fibres (Greenberg, 1958). Since 10 ml. of 10^{-16} M lysergic acid diethylamide contains about 600,000 molecules, this implies that an average of 6 molecules per cell is sufficient to drive many hearts at a maximum rate and amplitude for many hours. This assumes that all of the drug is adsorbed to the heart. That this is not the case was shown in a few experiments in which one heart was placed in a 10 ml. bath of 10^{-16} M lysergic acid diethylamide and allowed to remain there until maximally excited. This heart was

then removed and a second heart placed in the same fluid. The second heart was often strongly excited, indicating that the first heart had adsorbed only a fraction of the original number of molecules.

Gaddum & Paasonen (1955) made observations on a variety of molluscan hearts and found most of them to be excited by both 5-hydroxytryptamine and lysergic acid diethylamide. The heart beat of *Cardium edule*, however, was inhibited by the latter substance. Clearly one cannot generalize from the results with the *Venus* heart, nor can one apply the results from molluscan heart muscle to vertebrate smooth muscle where lysergic acid diethylamide generally antagonizes the action of 5-hydroxytryptamine (Gaddum & Hameed, 1954). Purpura (1956) has proposed that in the sensorimotor cortex of the cat, lysergic acid diethylamide inhibits at axodendritic synapses and facilitates at axosomatic synapses. Opposite actions of this substance at different sites within the same organism make it difficult to interpret its effects when administered to the whole animal.

On the *Venus* heart, (+)-lysergic acid derivatives without substituents at positions 1 or 2 had an excitor action resembling 5-hydroxytryptamine. These included the diethylamide, monoethylamide, propanolamide (ergometrine) and butanolamide (methylergometrine), also dihydroergotamine and lysergic acid itself. The four lysergic acid derivatives with substituents either in position 1 or 2 all showed weak excitor action or none at all, with varying degrees of specific inhibition of 5-hydroxytryptamine. That is, they antagonized in doses which had little or no excitor action. These were the 1-methyl-, 1-acetyl- and 2-bromo-derivatives of lysergic acid diethylamide, and methysergide. Of these, only the last failed to excite any heart in concentrations up to 10^{-4} M and after applications up to 4 hr in duration. It inhibited the responses to 5-hydroxytryptamine in a molar ratio of one to one and is the most effective antagonist of this neurotransmitter that has been found for the *Venus* heart. While this group of 5-hydroxytryptamine antagonists owe their actions in large part to substitutions in positions 1 or 2, the outstanding antagonistic action of methysergide may be attributed to the further presence of the butanolamide group.

After an extensive study of the structure-activity relations of a wide variety of tryptamine analogues on the *Venus* heart, Greenberg (1960) suggested that there are three negative binding sites for 5-hydroxytryptamine. It is suggested here that the amino-alcohol of lysergic acid derivatives provides a further important combining group, possibly to an adjacent 5-hydroxytryptamine receptor in the heart muscle. There is some indication that, in the *Venus* heart, acetylcholine receptor sites occur in pairs (Welsh & Taub, 1953). This may account, in part, for the effectiveness of benzoquinonium as an acetylcholine antagonist on this preparation, as first observed by Luduena & Brown (1952). The overlapping of a second adjacent 5-hydroxytryptamine receptor site by amino-alcohol or peptide derivatives of lysergic acid might account for their effectiveness as mimics or antagonists of 5-hydroxytryptamine.

The antagonism of 5-hydroxytryptamine by methysergide does not alter the response of the *Venus* heart to acetylcholine. This has been put to practical use in the assay of acetylcholine in tissue extracts that also contain 5-hydroxytryptamine.

Doepfner & Cerletti (1958) compared the effectiveness of a variety of lysergic acid derivatives and antihistamines as inhibitors of the oedema produced in the rat paw by injected 5-hydroxytryptamine. Methysergide was four times as effective as lysergic acid diethylamide in preventing the oedema produced by 5-hydroxytryptamine, and the more potent lysergic acid derivatives were 100 to 1,000 times more active than the most effective antihistamines. A more recent general account of the antagonism of 5-hydroxytryptamine by methysergide is that of Fanchamps, Doepfner, Weidmann & Cerletti (1960); this substance is a highly specific antagonist of 5-hydroxytryptamine in a variety of situations, including the rat uterus, the cardiovascular system and in 5-hydroxytryptamine toxicity.

Following the report by Sicuteri (1959) of the effectiveness of methysergide in preventing attacks of migraine, extensive clinical trials have been made by many workers with rather promising results.

In a study of the melanophore expanding effects of lysergic acid derivatives, Berde & Cerletti (1956) found 1-methyl-, 1-acetyl-, 2-bromo and 2-iodo-lysergic acid diethylamide to be among the most active in producing melanophore expansion in *Lebistes reticulatus*. Methysergide was not tested. The first three of these compounds are in the group having little excitor action but specific 5-hydroxytryptamine blocking action on the *Venus* heart. However, Berde & Cerletti observed lysergic acid diethylamide also to have a strong melanophore expanding action in *Lebistes*.

As noted above, the order of relative activities of the lysergic acid derivatives on the *Venus* heart is tentative, due, in part, to the difficulty of making repeated tests on a given heart. Even were this not the case, it would be unwise to generalize regarding their actions on other molluscan hearts, since it is already obvious from earlier studies that the pharmacology of the hearts of different classes and families of molluscs may show considerable variation.

This investigation was supported by Research Grant B-623 from the National Institute of Neurological Diseases and Blindness, U.S. Public Health Service.

The authors are indebted to Dr R. Bircher, Sandoz Pharmaceuticals, for generous gifts of chemicals and bibliographies.

REFERENCES

BACQ, Z. M., FISCHER, P. & GHIRETTI, F. (1952). Action de la 5-hydroxytryptamine chez les céphalopodes. *Arch. int. Physiol.*, **60**, 165–171.

BERDE, B. & CERLETTI, A. (1956). Über den Melanophoreneffekt von D-Lysergsäure-diäthylamid und verwandten Verbindungen. *Helv. Physiol. Acta*, **14**, 325–333.

DOEPFNER, W. & CERLETTI, A. (1958). Comparison of lysergic acid derivatives and antihistamines as inhibitors of the edema provoked in the rat's paw by serotonin. *Int. Arch. Allergy*, **12**, 89–97.

ERSPAMER, V. & GHIRETTI, F. (1951). The action of enteramine on the heart of molluscs. *J. Physiol. (Lond.)*, **115**, 470–481.

FANCHAMPS, A., DOEPFNER, W., WEIDMANN, H. & CERLETTI, A. (1960). Pharmacologische Charakterisierung von Deseril, einem Serotonin-Antagonisten. *Schweiz. med. Wschr.*, **90**, 1040–1046.

GADDUM, J. H. & HAMEED, K. A. (1954). Drugs which antagonize 5-hydroxytryptamine. *Brit. J. Pharmacol.*, **9**, 240–248.

GADDUM, J. H. & PAASONEN, M. K. (1955). The use of some molluscan hearts for the estimation of 5-hydroxytryptamine. *Brit. J. Pharmacol.*, **10**, 474–483.

GREENBERG, M. J. (1958). The action of indoles on the heart of *Venus mercenaria*. Ph.D. Thesis. Harvard Univ.

GREENBERG, M. J. (1960). Structure-activity relationship of tryptamine analogues on the heart of *Venus mercenaria*. *Brit. J. Pharmacol.*, **15**, 375–388.

KERKUT, G. A. & LAVERACK, M. S. (1960). A cardio-accelerator present in tissue extracts of the snail, *Helix aspersa*. *Comp. Biochem. Physiol.*, **1**, 62–71.

LOVELAND, R. E. (1962). Further evidence for a transmitter action of 5-hydroxytryptamine in the heart of *Mercenaria* (*Venus*) *mercenaria*. In preparation.

LUDUENA, F. P. & BROWN, T. G., Jr. (1952). Mytolon and related compounds as antagonists of acetylcholine on the heart of *Venus mercenaria*. *J. Pharmacol. exp. Therap.*, **105**, 232–239.

PURPURA, D. (1956). Electrophysiological analysis of psychotogenic drug action. II. General nature of lysergic acid diethylamide (LSD) action on central synapses. *Arch. Neurol. Psychiatry*, **75**, 132–143.

SHAW, E. & WOOLLEY, D. W. (1956). Some serotonin-like activities of lysergic acid diethylamide. *Science*, **124**, 121–122.

SICUTERI, F. (1959). Prophylactic and therapeutic properties of 1-methyl lysergic acid butanolamide in migraine. *Int. Arch. Allergy*, **15**, 300–307.

WELSH, J. H. (1953). Excitation of the heart of *Venus mercenaria*. *Arch. exp. Pathol. Pharmakol.*, **219**, 23–29.

WELSH, J. H. (1954). Hydroxytryptamine: A neurohormone in the invertebrates. *Fed. Proc.*, **13**, 162–163.

WELSH, J. H. (1957). Serotonin as a possible neurohumoral agent: Evidence obtained in lower animals. *Ann. N.Y. Acad. Sci.*, **66**, 618–630.

WELSH, J. H. & MCCOY, A. C. (1957). Action of d-lysergic acid diethylamide and its 2-bromo derivative on the heart of *Venus mercenaria*. *Science*, **125**, 348.

WELSH, J. H. & MOORHEAD, M. (1959). Identification and assay of 5-hydroxytryptamine in molluscan tissues by fluorescence method. *Science*, **129**, 1491–1492.

WELSH, J. H. & MOORHEAD, M. (1960). The quantitative distribution of 5-hydroxytryptamine in the invertebrates, especially in their nervous systems. *J. Neurochem.*, **6**, 146–169.

WELSH, J. H. & TAUB, R. (1948). The action of choline and related compounds on the heart of *Venus mercenaria*. *Biol. Bull.*, **95**, 346–353.

WELSH, J. H. & TAUB, R. (1953). The action of acetylcholine antagonists on the heart of *Venus mercenaria*. *Brit. J. Pharmacol.*, **8**, 327–333.