EFFECT OF 5-HYDROXYTRYPTAMINE AND RELATED COMPOUNDS ON THE ISOLATED HEART OF *PILA GLOBOSA* (GASTROPODA: MOLLUSCA)

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ABSTRACT

5-hydroxytryptamine produces an increase in the amplitude and frequency of the heart-beat of *Pila.* The threshold for the activity of 5-hydroxytryptamine is 10^{-8} gm./ml. However, this dose is not constant. In some cases it is 10^{-6} gm./ml. and in still other cases doses even upto 10^{-4} gm./ml. are ineffective. The action of 5-HT is not blocked by LSD. However, LSD 10^{-5} gm./ml. produces a positive inotropic and a very slight positive chronotropic response. The bromine derivative of LSD sometimes causes a rise in the threshold of 5-HT. Reserpine produces a positive inotropic and a negative chronotropic effect. The threshold dose for Reserpine is 10^{-5} gm./ml. The action of Reserpine is of long duration and both increase in amplitude and decrease in frequency go on for a long time in a progressive manner. The action of 5-HT and related compounds on the heart of *Pila* has been discussed in relation to that in other molluscs.

DURING recent years 5- Hydroxytryptamine has gained sufficient importance because of its extensive occurrence in body tissues and its reported activity on the smooth muscles and central nervous system of mammals. Welsh (1953, 1954, 1956, 1957) and Welsh and McCoy (1957) have shown the possible role of this substance in the working of the heart in molluscs.

The methods for maintaining the heart of *Pila in vitro* and the recording of the heart-beat have been described earlier (Lal and Agarwal, 1967). For the purpose of studying the effect of this drug on the heart, 5-hydroxy-tryptamine creatinine sulphate (Serotonin) was used. The concentrations of 5-HT referred to in the following pages are actually those of this substance. Solution of Serotonin were prepared fresh everyday. Besides this, the action of LSD, Bromo-LSD and Reserpine on the heart of *Pila* were also noted.

5-HT has been found to stimulate the heart of *Pila* at very low concentrations. The threshold for 5-HT action lies at 10^{-8} gm./ml. (Fig. 1). At this concentration 5-HT raises the amplitude of the heart-beat of *Pila*. At higher concentrations (10^{-7} gm./ml. and above) there is a rise in both amplitude and frequency of the heart-beat of *Pila* (Fig. 2). A change in tone is generally absent though in a few cases there may be a slight rise of the tone also. Although in most cases there is a rise in frequency, the rise in amplitude is always present and is the more dependable factor.

The sensitivity of the heart of *Pila* to 5-HT was not found to be uniform in all the preparations. In some of the preparations the threshold was found to be 10^{-6} gm./ml., whereas in others even 10^{-4} gm./ml. failed to evoke any response. This variation is found not only in the threshold dose but also in the magnitude of response which the same dose of 5-HT produces in different individuals whose threshold is the same. There is, however, no doubt regarding the sensitivity of the heart of *Pila* towards 5-HT at low concentrations. It is not clear why 5-HT gets so curiously blocked in some hearts.

The action of 5-HT is of long duration but the maximal effect is reached within 5 to 7 minutes (Figs. 1, 2). There is also an immediate drop in the frequency level after washing.

It was found that the sensitivity of heart towards 5-HT decreased with successive doses but it was difficult to say that it was due to tachyphylaxis as reported by Greenberg (1960) as in *Pila*, 5 HT does not have a uniform effect like other drugs such as acetylcholine, adrenaline and nor-adrenaline. In fact due to this fluctuation of the threshold of 5-HT this study becomes difficult. Moreover, in many cases 5-HT does not appear to have an effect at all and is blocked by the heart itself. Gaddum and Paasonen (1955) have reported similar fluctuation of the effect of 5-HT on the heart of *Spisula*.

The effect of Lysergic acid diethylamide (LSD) which closely mimics the action of 5-HT (Shaw and Wooley, 1956 ; Welsh and McCoy, 1957 ; Welsh, 1957) was also studied. Stock solutions of LSD were prepared in halogen free water. However, when these solutions were added to the bath full of saline, LSD came in contact with halogens present in the saline but it was found that LSD remained active in it for a long time in order to create its maximal effect. LSD 10^{-5} gm./ml. produced a positive inotropic effect and a very slight positive chronotropic effect (Fig. 3). The amplitude goes on increasing consistently and the maximal effect is attained in about

12 minutes. Recovery after washing is not as quick as in the case of 5-HT although the amplitude starts falling off soon after washing.

The bromine derivative of LSD, Bromo-LSD (BOL) apparently does not have any action even in doses ten times higher than that of LSD (Fig. 5). In *Pila,* however, it has been observed that after the administration of Bromo-LSD the threshold of the action of 5-HT on the heart of *Pila* is raised. As has been mentioned earlier the action or range of the action of 5-HT on the heart of *Pila* is quite variable and since it has been found to change in the same experiment even when nothing is added from outside it becomes difficult to say whether the raising of threshold of 5-HT is really due to Bromo-LSD.

In recent years Reserpine has been reported by Pletscher, Shore and Brodie (1956), Shore, Silver and Brodie (1955) and Brodie, Pletscher and Shore (1956) to affect the 5-HT metabolism in the brain of mammals to a great extent.

Reserpine had a positive inotropic and a negative chronotropic effect on the heart of *Pila.* The threshold dose for Reserpine was 10^{-5} gm./ml. in the case of *Pila* (Fig. 5). In *Pila* (like mammals) this drug had a prolonged action. It was observed to increase the amplitude for more than an hour after the drug had been administered (Fig. 5).

As was expected with a long acting drug the recovery after washing was very slow although it started appearing soon after washing.

In one experiment it was also found that Reserpine produced a sort of *arrhythmia* in the heart. In this case although there was a progressive increase in the amplitude there were 2–3 short beats in between two complete contractions (Fig. 6).

Both decrease in frequency and increase in amplitude caused by Reserpine continued in a progressive manner and it was found that by the end of an hour the increase in amplitude was about 50 per cent. with a Reserpine dose of 10^{-5} gm./ml. at comparatively higher doses of Reserpine ($2 \times 10^{-5} + 2 \times 10^{-5}$ gm./ml.) there was a rise in the tone as well (Fig. 7).

Discussion

5-Hydroxytryptamine is an important neurohormone reported from the central nervous system of vertebrates. In many molluscs this drug has been shown to have a stimulating effect on the heart. It stimulates the heart of *Venus* (Welsh, 1954 ; 1957 ; Greenberg, 1960 *a* and 1960 *b*), *Anodonta*

(Fange, 1955), *Cyprina* (Welsh, 1956), *Mya*, *Spisula* and *Helix* (Gaddum and Paasonen, 1955), *Buccinum* (Gaddum and Paasonen, 1955 ; Welsh 1953 and 1956), *Busycon* and *Strombus* (Hill, 1958). Mostly 5-HT manifested its excitatory action in the form of positive inotropic and positive chronotropic effects. In *Buccinum*, however (Welsh, 1954), there is a positive tonotropic effect as well. In most of the forms the range of activity of 5-HT is quite large and even high doses of the drug fail to stop the heart, *e.g.*, in *Busycon* where the threshold is 10^{-1}, a dose of 10^{-2} does not produce cardiac arrest (Hill, 1958). In most of the forms there is a graded response with the change of the dose. The threshold for *Anodonta* is 10^{-9} (Fange, 1955), for *Cyprina* 10^{-10} (Welsh, 1954), *Venus* 10^{-10} to 10^{-9} (Greenberg, 1960 *a*) *Busycon* 10^{-9} and *Strombus* 10^{-10} (Hill, 1958) and *Buccinum* 10^{-10} (Welsh, 1953). The threshold for *Mya* is 5×10^{-7}, for *Spisula* 10^{-7} and for *Helix* 10^{-6} (Gaddum and Paasonen, 1955). In some forms the threshold is not constant as is the case with *Pila*. In *Buccinum* where the threshold is 10^{-10} as reported by Welsh (1953), and 10^{-6} as reported by Gaddum and Paasonen (1955) even doses upto 10^{-4} are sometimes inactive (Gaddum and Paasonen, 1955).

LSD stimulates the hearts of several molluscs. Previously it was reported by Welsh that LSD blocked 5-HT in the heart of *Venus* (Welsh, 1954), *Cyprina* and *Buccinum* (Welsh, 1954 ; 1956) but later on it was found by Welsh himself (Welsh, 1957) that LSD did not block 5-HT in *Venus*, but instead had an excitor effect of its own. In this action LSD closely mimics the action of 5-HT (Welsh, 1957). This finding has further been confirmed by Shaw and Wooley (1956) who stated that the action of LSD is like that of Serotonin. In *Cyprina* and *Buccinum* also this substance does not block 5-HT, neither does it have a very significant excitatory action of its own (Welsh, 1957).

The bromine derivative of LSD sometimes caused a rise in the threshold of action of 5-HT in case of *Pila*.

In *Pila* both 5-HT and LSD have a stimulating effect.

Reserpine is an alkaloid whose effect has not been studied so far in the molluscs. This alkaloid has the property of releasing Serotonin from the body tissue (Pletscher, Shore and Brodie, 1956), and from the blood platelets (Haverback, Shore, Tomich and Brodie, 1956). In man, it produces a slowing of the heart (Goodman and Gillman, 1960). In *Pila* also it was found that Reserpine reduced the frequncy of heart-beat. The increase in amplitude of the heart may be due to the release of 5-HT from the heart

tissue and 5-HT may be acting as a neurohormone in the case of this mollusc.

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EXPLANATION OF PLATE VIII

FIG. 1. Graph showing the positive inotropic effect produced by 5–HT 10^{-8} gm./ml. on the heart of *Pila globosa*. W=wash; Time scale = 2 minutes.

FIG. 2. Graph showing the positive inotropic, positive chronotropic and positive tonotropic effects produced by 5–HT 10^{-7} gm./ml. on the heart of *Pila globosa*. W = wash; Time scale = 2 minutes.

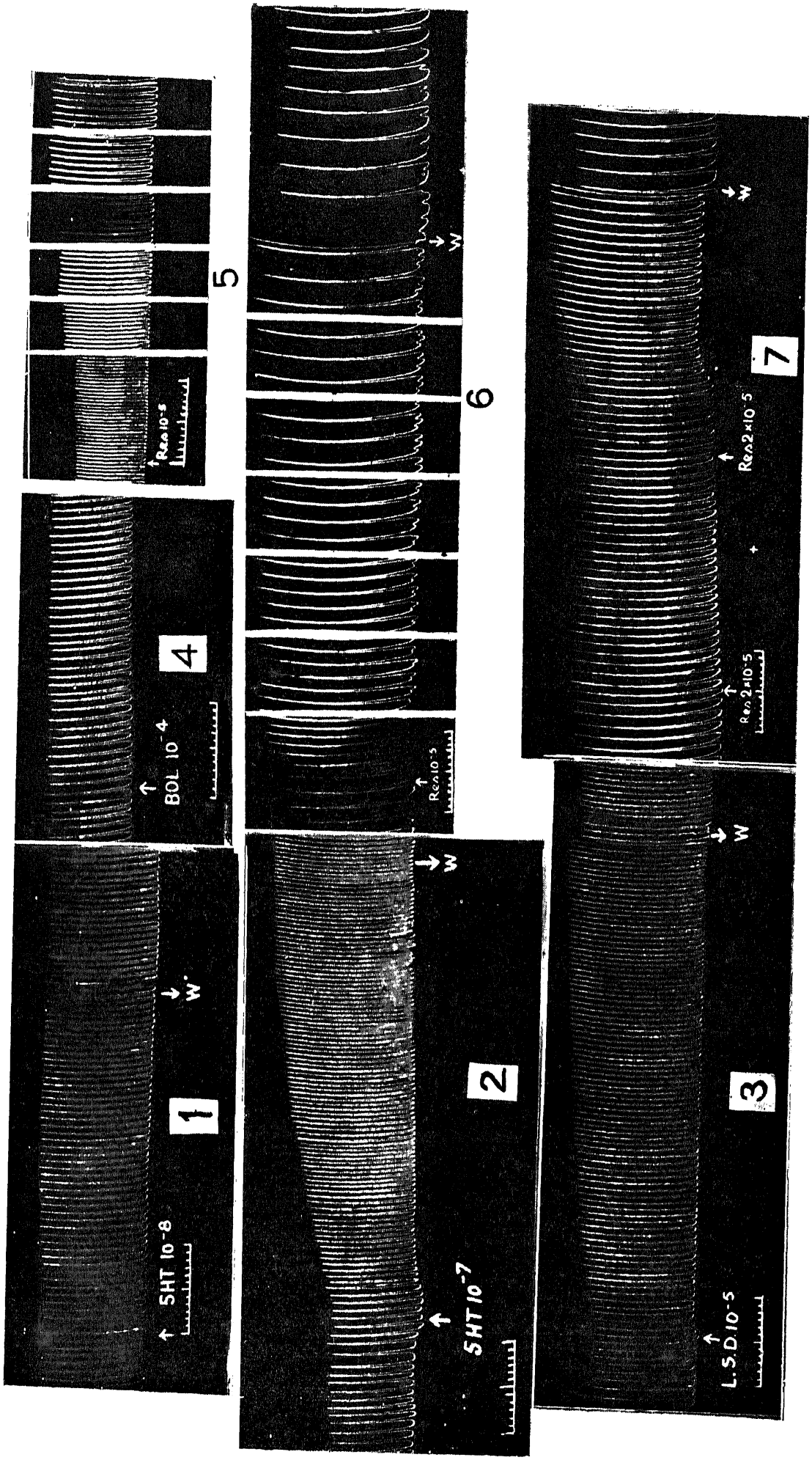
FIG. 3. Graph showing the positive inotropic and positive chronotropic effects produced by LSD 10^{-5} gm./ml. on the heart of *Pila globosa*. W = wash; Time scale = 2 minutes.

FIG. 4. Graph showing that Bromo–LSD (BOL) 10^{-4} gm./ml. has no effect on the heart of *Pila globosa*. W = wash; Time scale = 2 minutes.

FIG. 5. Graph showing the positive inotropic and negative chronotropic effects produced by Reserpine 10^{-5} gm./ml. over a period of one hour on the heart of *Pila globosa*. One minute records taken at 9 minutes interval. Time scale = 2 minutes.

FIG. 6. Graph showing the positive inotropic, negative chronotropic effects and arrhythmia produced by Reserpine 10^{-5} gm./ml. over a period of one hour on the heart of *Pila globosa*. 1·5 minutes records taken at 8 minutes interval. W = wash; Time scale = 2 minutes.

FIG. 7. Graph showing the positive tonotropic effect produced by two successive doses of Reserpine 2×10^{-5} gm./ml. on the heart of *Pila globosa*. W = wash; Time scale = 2 minutes.


1
SHT 10⁻⁸
W
2
SHT 10⁻⁷
W
3
L.S.D.10⁻⁵
W
4
BOL 10⁻⁴
W
5
Re 10⁻⁵
6
Re 10⁻⁵
W
7
Re 2·10⁻⁵
Re 2·10⁻⁵
W

FIGS. 1–7