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The 5-hydroxytryptamine-like actions of 5,6-dihydroxytryptamine

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Summary

1. With isolated preparations of rat stomach fundus as well as of duodenum and ileum of rats and guinea-pigs, 5,6-dihydroxytryptamine and 5,6-diacetoxytryptamine caused a contraction which was antagonized by methysergide and lysergic acid diethylamide (LSD), but not by atropine. Pretreatment of the animals with reserpine did not decrease the effect of the two indoleamines on the isolated ileum and duodenum.

2. In anaesthetized guinea-pigs, 5,6-dihydroxytryptamine and its diacetyl derivative caused bronchoconstriction which was antagonized by methysergide, but not modified by pretreating the animals with reserpine.

3. In anaesthetized cats, 5,6-dihydroxytryptamine had, in general, a hypotensive effect which was reversed by hexamethonium.

4. 5,6-Dihydroxytryptamine also caused aggregation of isolated rabbit and human platelets and inhibited the platelet aggregation induced by 5-hydroxytryptamine (5-HT) plus adrenaline.

5. The pattern of action of 5,6-dihydroxytryptamine and 5,6-diacetoxytryptamine was qualitatively the same as that of 5-HT, but the potency of the compounds decreased in the order 5-HT, 5,6-dihydroxytryptamine, 5,6-diacetoxytryptamine both *in vitro* and *in vivo*.

6. It is concluded that 5,6-dihydroxytryptamine and its diacetyl derivative stimulate postsynaptic 5-HT receptors, but that their effect is weaker than that of 5-HT.

Introduction

Injection of 5,6-dihydroxytryptamine into the lateral cerebral ventricles of rats causes a long-lasting, selective diminution of the concentration of 5-hydroxytryptamine (5-HT) in the central nervous system (Baumgarten, Björklund, Lachenmayer, Nobin &Stenevi, 1971 ; Baumgarten, Evetts, Holman, Iversen, Vogt & Wilson, 1972b ; Costa, Lefevre, Meek, Revuelta, Spano, Strada & Daly, 1972 ; Da Prada, Carruba, O'Brien, Saner & Pletscher, 1972). This decrease has been shown to be due to a relatively selective chemical degeneration of indoleamine-containing nerve terminals in the rat brain (Baumgarten, Björklund, Holstein & Nobin, 1972a ; Baumgarten & Lachenmayer, 1972 ; Baumgarten, Lachenmayer & Schlossberger, 1972d). Thus, the effect of 5,6-dihydroxytryptamine on 5-hydroxytryptaminergic neurones seems to be analogous to that of 6-hydroxydopamine on catecholaminergic nerve terminals (Thoenen & Tranzer, 1968 ; Tranzer & Thoenen,

1968). 5,6-Dihydroxytryptamine also acts on extracerebral cells, e.g. on blood platelets in which it interferes with the uptake, storage and degradation of 5-HT (Da Prada, O'Brien, Tranzer & Pletscher, 1973).

The action of 5,6-dihydroxytryptamine on receptors sensitive to 5-HT has not yet been studied extensively. Recently, it has been reported that in pithed rats, 5,6-dihydroxytryptamine acted like 5-HT by directly stimulating 5-HT receptors, leading to an increase in blood pressure (Baumgarten, Göthert, Schlossberger & Tuchinda, 1972c). In the present work, these investigations were extended by studying the action of 5,6-dihydroxytryptamine and its diacetyl derivative on various 5-HT-sensitive tissues *in vitro* and *in vivo*.

Methods

Isolated gastric strips, duodenum and ileum

Male Sprague-Dawley rats (250–300 g) and male guinea-pigs (350–450 g) were fasted for 12 h before decapitation. The rat stomach strips were prepared according to the method of Vane (1957), the guinea-pig ileum and rat duodenum were isolated using conventional methods.

Each preparation was suspended in a bath containing 10 ml of Tyrode solution gassed with a mixture of O_2 (95%) and CO_2 (5%) at a temperature of 37° C. Isometric contractions were recorded by means of a microdynamometer (Basile S.p.A., Milan) consisting of a transducer connected to an amplifier and a writing device. The test substances, i.e. 5,6-dihydroxytryptamine, 5,6-diacetoxytryptamine (both synthesized by Dr. A. Kaiser, Chemical Research Department, F. Hoffmann-La Roche & Co. Ltd., Basel) and 5-HT (Vister), all as creatinine sulphates, as well as antagonists such as methysergide, lysergic acid diethylamine (LSD) (both Sandoz, Basel) and atropine sulphate (B.D.H.), were dissolved in 0·9% w/v NaCl solution containing 0·01% ascorbic acid and added to the bath in a volume of 0·1–0·5 ml. At 5-min intervals, the isolated tissue was exposed to the agonist substances for a period of 30 s ; the preparation was then washed with Tyrode solution. The final concentration of the compounds in the bath are expressed as μM. Iproniazid (Roche ; 80 mg/kg i.p.) and reserpine (Ciba ; 10 mg/kg s.c.) were administered to rats 18 h prior to killing.

Blood pressure and heart rate

Cats, fasted for 12 h and anaesthetized with chloralose (80 mg/kg i.v.), were tracheotomized and an endotracheal tube was inserted. The test substances including hexamethonium (Sigma, 10 mg/kg) were injected into the femoral vein. The arterial pressure was recorded from the femoral artery with a pressure transducer. Heart frequency was measured with a cardiotachometer and respiration by a pressure transducer connected to the endotracheal cannula. These parameters were recorded on a ' Battaglia-Rangoni ' polygraph.

Bronchial resistance in guinea-pigs

Male guinea-pigs, weighing 600 g, were anaesthetized with urethane (1·5 g/kg i.p.) after a fasting period of 12 hours. Bronchial resistance was measured by the method of Konzett & Rössler (1940) modified by Rosenthale & Dervinis (1968).

The trachea was cannulated and connected to a 'Miniature Starling Palmer' pump which supplied to the lung a constant volume (10 ml) of air at a frequency of 72 strokes per minute. The expired air displaced a piston in a water manometer connected to a transducer and charge recorder. All test substances were injected into the jugular vein.

Reserpine (10 mg/kg) was given subcutaneously to guinea-pigs 18 h prior to killing.

Aggregation of blood platelets

Blood samples were collected from the carotid artery of 8 rabbits and the cubital vein of a human volunteer, and platelet-rich plasma was prepared as described earlier (Baumgartner, 1969 ; Baumgartner & Born, 1968).

Platelet aggregation in stirred plasma was measured by a photometric method (Born, 1962) and expressed as aggregation velocity in mm/min ; the recorder scale was calibrated as previously described (Baumgartner & Born, 1968, 1969). The aggregating agents were 5-HT creatinine sulphate (Sigma), (−)-adrenaline-dihydrogen tartrate (Fluka) and 5,6-dihydroxytryptamine. They were dissolved in 0·9% w/v NaCl solution containing 0·1% ascorbic acid and added to platelet-rich plasma (Baumgartner & Born, 1968, 1969) to give the final plasma concentrations indicated below.

Results

Isolated fundus of rat stomach

5,6-Dihydroxytryptamine caused a contraction of the isolated fundus of rat stomach. This effect increased with increasing concentrations of the compound. The dose-response curve of 5,6-dihydroxytryptamine was parallel to that of 5-HT However, the ED50 of 5,6-dihydroxytryptamine was almost 10 times higher than that of 5-HT (Fig. 1). 5,6-Diacetoxytryptamine at twice the molar concentration of 5,6-dihydroxytryptamine showed an effect equal to that of the non-acetylated compound.

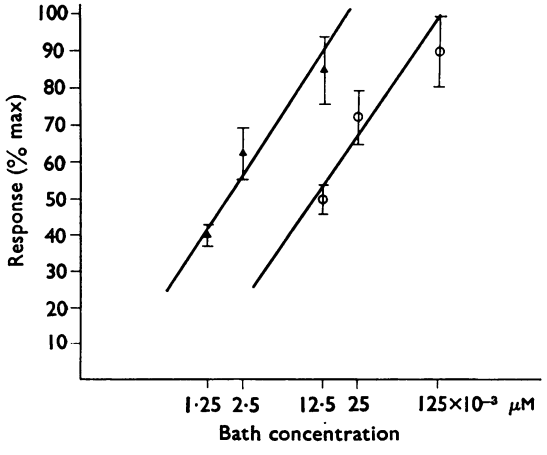

FIG. 1. Isolated stomach fundus of rat. Dose-response curves to 5-hydroxytryptamine (▲) and 5,6-dihydroxytryptamine (○). Mean ± S.E.M. of 6 experiments.

Methysergide (0·1 μM) abolished the contractions induced by 5,6-dihydroxytryptamine ($1{\cdot}25\times10^{-1}$ μM), 5,6-diacetoxytryptamine ($2{\cdot}5\times10^{-1}$ μM) and 5-HT ($1{\cdot}25\times10^{-2}$ μM) (Fig. 2). A similar effect was seen with LSD (0·2 μM). In contrast, atropine (15 μM) slightly potentiated the stimulatory effects of 5-HT, 5,6-dihydroxytryptamine and 5,6-diacetoxytryptamine.

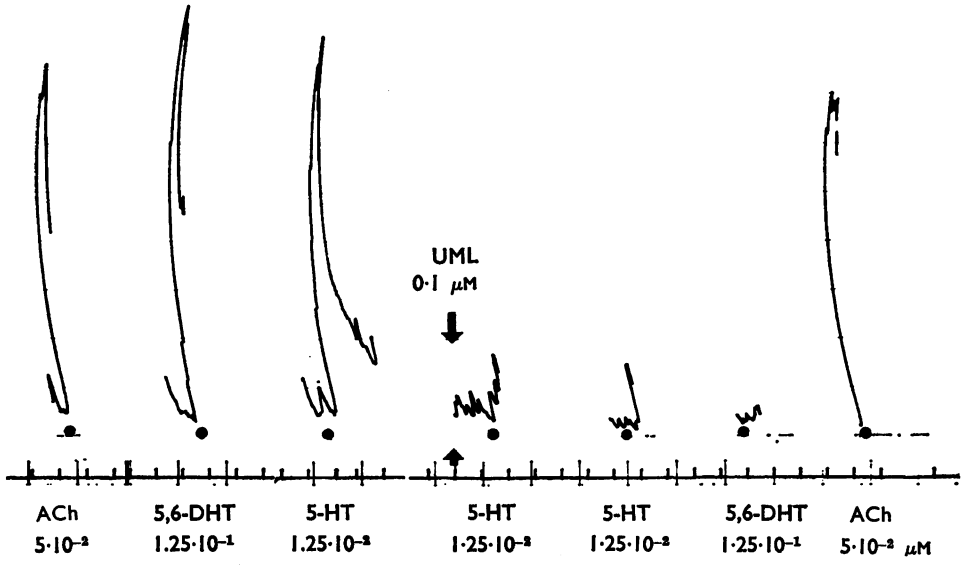

FIG. 2. Isolated stomach fundus of rat. Effects of methysergide (UML) on the contraction induced by acetylcholine (ACh), 5-hydroxytryptamine (5-HT) and 5,6-dihydroxytryptamine (5,6-DHT).

Pretreatment of the rats with an i.p. injection of iproniazid, an inhibitor of monoamine oxidase, did not increase the response of the fundus preparation to 5,6-dihydroxytryptamine or (in confirmation of earlier findings (Vane, 1959)) to 5-HT.

Isolated guinea-pig ileum and rat duodenum

Both these tissues reacted to 5-HT in a way similar to the isolated stomach fundus. The responses of the intestinal preparations to 5,6-dihydroxytryptamine and 5,6-diacetoxytryptamine were qualitatively similar to those obtained with 5-HT. Although a thorough dose-response study was difficult to perform because of tachyphylaxis, 5,6-dihydroxytryptamine and 5,6-diacetoxytryptamine showed 5–10 times and 10–20 times, respectively, less activity than 5-HT.

Intestinal preparations obtained from animals pretreated with reserpine were as sensitive to 5,6-dihydroxytryptamine and 5,6-diacetoxytryptamine as preparations from untreated animals.

Blood pressure in cats

Injection of 50–200 μg/kg of 5,6-dihydroxytryptamine caused, in most instances a rapid, dose-dependent decrease of blood pressure which was followed by a return to normal after 2–5 minutes. 5-HT, which induced a similar fall in blood pressure,

was, however, 5–6 times more effective than 5,6-dihydroxytryptamine (Fig. 3). In rare instances, the two indoleamines caused a blood pressure rise. In cats pretreated with hexamethonium (15 min before the indoleamines), 5,6-dihydroxytryptamine, as well as 5-HT, always had a rapid, short-lasting (2–5 min) hypertensive action (Fig. 4) confirming earlier findings with 5-HT (Page & McCubbin, 1953; Schneider & Yonkman, 1954).

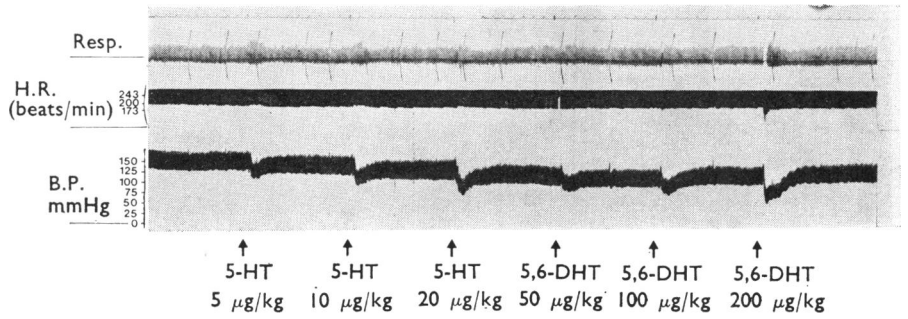

FIG. 3. Effects of increasing i.v. doses of 5-hydroxytryptamine (5-HT) and 5,6-dihydroxytryptamine (5,6-DHT) on cat respiration (Resp.), heart rate (H.R.) and blood pressure (B.P.).

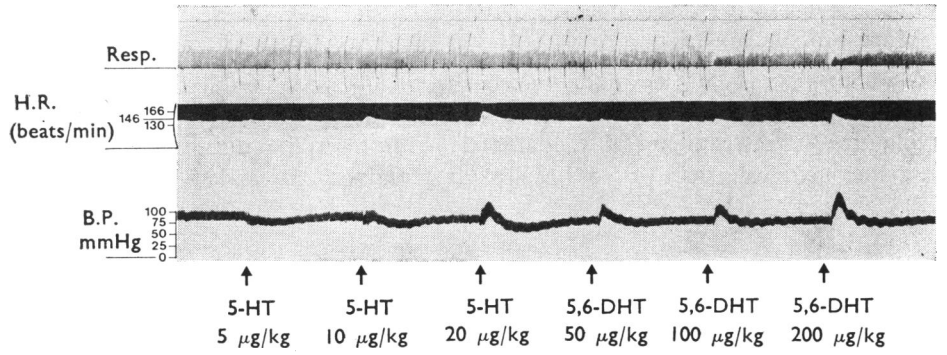

FIG. 4. Effects of increasing i.v. doses of 5-hydroxytryptamine (5-HT) and 5,6-dihydroxytryptamine (5,6-DHT) on respiration (Resp.), heart rate (H.R.) and blood pressure (B.P.) of cat pretreated with hexamethonium (10 mg/kg i.v. 15 min before injection of the indolealkylamines).

5,6-Diacetoxytryptamine, which had effects on blood pressure similar to those of 5,6-dihydroxytryptamine and 5-HT, was, however, 2–4 times less active than 5,6-dihydroxytryptamine.

Bronchial resistance in guinea-pigs

Low doses of 5,6-dihydroxytryptamine (2·5–10 μg/kg) had no effect in some animals, whereas in others the compound produced either bronchoconstriction or mild bronchodilatation. Higher doses of 5,6-dihydroxytryptamine (>10 μg/kg) always caused a bronchoconstriction which was dose-dependent. 5-HT also had a bronchoconstrictor action which increased with increasing doses of the amine (Fig. 5). 5,6-Dihydroxytryptamine was approximately 5–10 times less effective than 5-HT. 5,6-Diacetoxytryptamine, which had an effect similar to the other indole-

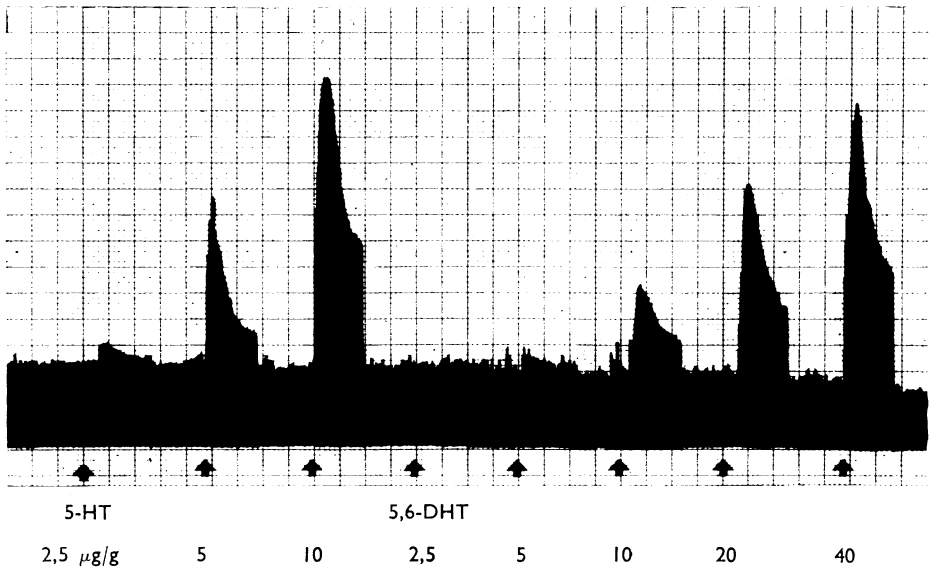

FIG. 5. Guinea-pig bronchospasm. Effect of increasing i.v. doses of 5-hydroxytryptamine (5-HT) and 5,6-dihydroxytryptamine (5,6-DHT).

amines, was, however, less potent. Doses as high as 40 μg/kg were required to produce bronchoconstriction.

Methysergide (1 mg/kg) antagonized the 5-HT- and 5,6-dihydroxytryptamine-induced bronchospasm (Fig. 6) and also counteracted the effect of 5,6-diacetoxytryptamine. Pretreatment of the animals with reserpine did not influence the bronchoconstrictor effect of 5,6-dihydroxytryptamine or its diacetyl derivative.

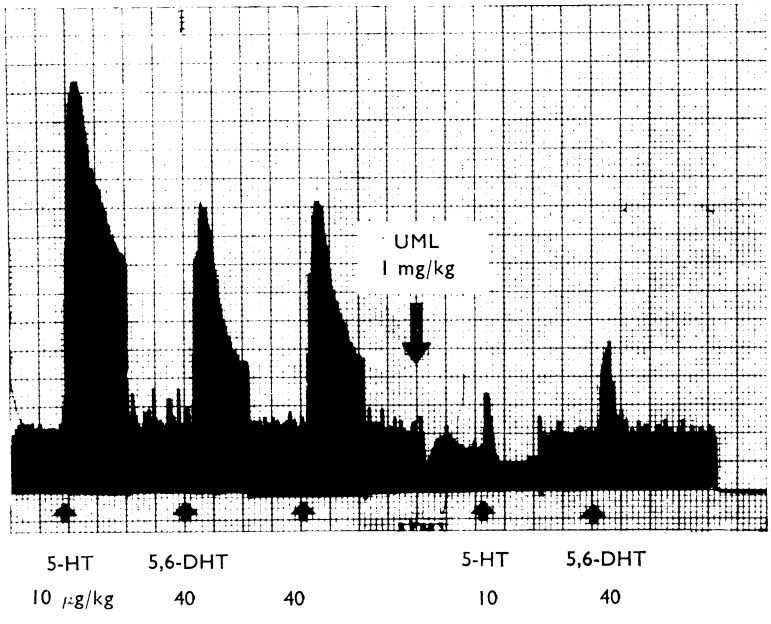

FIG. 6. Effect of methysergide (UML 1 mg/kg i.v.) on the 5-hydroxytryptamine (5-HT)- and 5,6-dihydroxytryptamine (5,6-DHT)-induced bronchospasm in guinea-pigs.

Platelet aggregation

5,6-Dihydroxytryptamine caused shape change of rabbit platelets followed by aggregation which showed maximal values after 30 s and was completely reversed after 60 seconds. In the lower concentration range of 5,6-dihydroxytryptamine, the aggregation velocity increased with increasing concentrations of the compound. Aggregation reached a maximum with about 100 μM 5,6-dihydroxytryptamine and progressively decreased at higher concentrations. 5,6-Dihydroxytryptamine, 1,000 μM, caused platelet shape change, but no aggregation. 5-HT produced responses similar to those caused by 5,6-dihydroxytryptamine, but maximal aggregation velocity was reached with 10 μM. However, maximal aggregation velocity induced by 5,6-dihydroxytryptamine was of approximately the same magnitude as that caused by 5-HT (Figure 7). Adrenaline, which by itself does not aggregate rabbit platelets (Baumgartner, 1969), enhanced the aggregation due to 5,6-dihydroxytryptamine as well as that induced by 5-HT. Experiments with human platelet-rich plasma gave similar results.

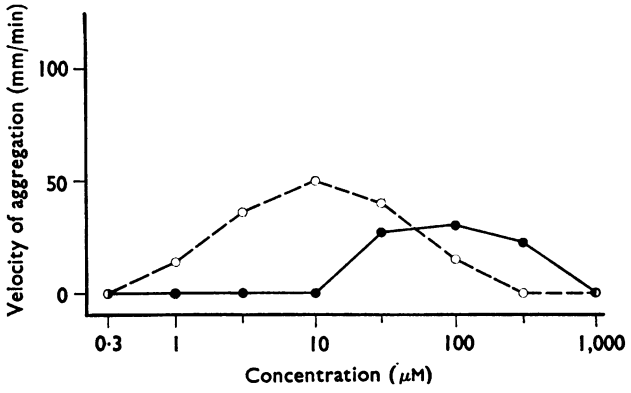

FIG. 7. Platelet aggregation velocities produced by different concentrations of 5-hydroxytryptamine ○- - -○ and 5,6-dihydroxytryptamine ●——● in platelet-rich plasma of a rabbit. Similar results were obtained in 4 other experiments.

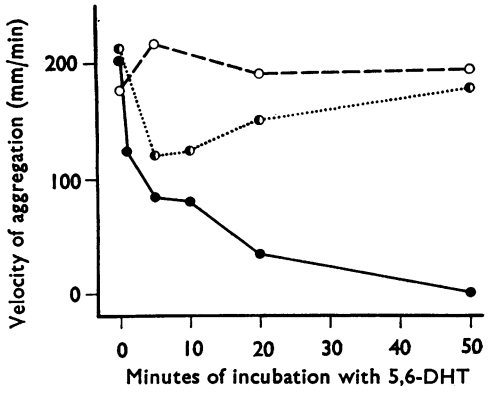

FIG. 8. Inhibition of the aggregation of rabbit platelets by incubation with 5,6-dihydroxytryptamine (5,6-DHT). Three samples of platelet-rich plasma were incubated at 37° C with 0·9% w/v NaCl solution ○- - -○, 5,6-dihydroxytryptamine (5 μM) ◐· · ·◐ or 5,6-dihydroxytryptamine (50 μM) ●——●. At the time intervals indicated, aggregation was produced by 5 μM 5-hydroxytryptamine plus 5 μM adrenaline. Similar results were obtained in 2 other experiments.

Incubation of platelet-rich plasma with 5 μM 5,6-dihydroxytryptamine for 10 min diminished platelet aggregation induced by 5-HT plus adrenaline. When the incubation period was prolonged, the aggregation velocity of the platelets gradually returned to control values after 50 minutes. Incubation of the platelets with higher concentrations of 5,6-dihydroxytryptamine (50 μM) caused an inhibition of the aggregation which progressed with increasing incubation time and was complete after 50 min (Figure 8).

Discussion

In the experiments described here, 5-HT showed the well-known pharmacological effects, i.e. contraction of isolated gastrointestinal preparations (Erspamer, 1940, 1966 ; Gaddum, 1953 ; Vane, 1957), arterial hypotension in anaesthetized cats (in rare instances hypertension) (Page & McCubbin, 1953 ; Schneider & Yonkman, 1954 ; Erspamer, 1966), reversal of this hypotensive effect by hexamethonium (Page & McCubbin, 1953 ; Schneider & Yonkman, 1954), and bronchospasm in guinea-pigs (Konzett, 1956 ; Erspamer, 1966). In addition, the present results demonstrate that 5,6-dihydroxytryptamine and 5,6-diacetoxytryptamine mimic the action of 5-HT in all the systems mentioned. The effects of the first two compounds, like those of 5-HT (Erspamer, 1966), were counteracted by 5-HT antagonists such as methysergide and LSD. This indicates that 5,6-dihydroxytryptamine and 5,6-diacetoxytryptamine stimulate 5-HT receptors either directly or indirectly, i.e. via release of 5-HT from its storage depots. The experiments with reserpine are in favour of a direct receptor stimulation. Thus, the contraction of the isolated duodenum and ileum as well as the bronchoconstriction induced by 5,6-dihydroxytryptamine and its diacetyl derivatives was not diminished after reserpine pretreatment of the animals, although reserpine has been shown virtually to deplete the 5-HT stores of intestine and lungs (Waalkes, Coburn & Terry, 1959).

5,6-Dihydroxytryptamine also mimicked the action of 5-HT on isolated platelets. Like 5-HT and other 5-hydroxy- and 5-methoxy-indolealkylamines (Born, Juengjaroen & Michal, 1972), it caused aggregation and/or swelling of isolated platelets of rabbits and man. Furthermore, incubation with 5,6-dihydroxytryptamine also inhibited the platelet aggregation induced by 5-HT plus adrenaline. A similar inhibition of this aggregation occurred when platelets were preincubated with 5-HT (Baumgartner, 1969). In both the direct aggregation effect and the inhibition of the 5-HT plus adrenaline-induced aggregation, 5,6-dihydroxytryptamine was less potent than 5-HT.

It has been proposed that the inhibition of the platelet aggregation caused by 5-HT is due to a saturation of the transport and storage system of the platelets with this amine (Baumgartner, 1969 ; Baumgartner & Born, 1969). The inhibitory effect of 5,6-dihydroxytryptamine may result from a similar saturation process. In fact, it has been demonstrated that 5,6-dihydroxytryptamine is taken up by isolated platelets and accumulates in their storage organelles (Da Prada *et al.*, 1973).

The present experiments confirm and expand the findings obtained in pithed rats (Baumgarten *et al.*, 1972c). The fact that in the latter species, 5,6-dihydroxytryptamine caused arterial hypertension, whereas in anaesthetized cats hypotension predominated is not surprising, since 5-HT showed the same differences in these two species (Page & McCubbin, 1953 ; Schneider & Yonkman, 1954 ; Salmoiraghi,

Page & McCubbin, 1956; Erspamer, 1966; Baumgarten *et al.*, 1972c). The above results also indicate that introduction of an OH-group in 6-position of the 5-HT molecule weakens the receptor stimulant effect. Acetylation of the two aromatic OH-groups of 5,6-dihydroxytryptamine which causes chemical stabilization of the molecule further decreases its biological activity.

It may be concluded that in peripheral tissues 5,6-dihydroxytryptamine and its diacetyl derivative not only act on the transport, storage and metabolism of 5-HT, but are also 5-HT receptor agonists, though their action is less potent than that of 5-HT *in vitro* and *in vivo*. Whether 5,6-dihydroxytryptamine has an effect on 5-HT receptors besides its action on 5-hydroxytryptaminergic nerve terminals in the central nervous system remains to be elucidated.

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