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5-HYDROXYTRYPTAMINE AND TRANSMISSION IN SYMPATHETIC GANGLIA

BY

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Rat stellate ganglia were isolated and maintained in a moist chamber in which they were intermittently immersed in physiological saline containing low concentrations of the compound under investigation. 5-Hydroxytryptamine (serotonin) in concentrations as low as 1.3×10^{-6} M reduced reversibly the threshold and increased the amplitude of responses to preganglionic stimulation. Certain antagonists of 5-hydroxytryptamine depressed reversibly the postsynaptic responses. Experiments *in vivo* supported this finding. It is suggested that 5-hydroxytryptamine facilitates transmission in the sympathetic ganglia of rats.

The effect of 5-hydroxytryptamine on transmission of nerve impulses in the autonomic nervous system has been explored by several investigators. Marrazzi & Hart (1953) reported that, while 65 μg/kg of 5-hydroxytryptamine had no effect on nonsynaptic potentials, it blocked reversibly ganglionic transmission in C fibres of the ciliary ganglion preparation of the dog. Douglas & Ritchie (1957) observed a decline in potentials evoked antidromically in C fibres of the vagus nerve of rabbits after intravenous injection of 5-hydroxytryptamine (10 to 100 μg) and concluded that spontaneous activity of the fibres was thereby increased. Marrazzi (1957) reported that 5-hydroxytryptamine induced synaptic inhibition in transcallosal pathways in the cat (10 μg/kg).

Trendelenburg (1957) showed that intra-arterial injections of 5-hydroxytryptamine potentiated the response of the cat nictitating membrane to submaximal stimulation of the cervical sympathetic trunk and concluded that this was due to an action of the compound upon the ganglion cells although the possibility of an effect on the end organ could not be excluded. Bindler & Gyermek (1961) found that 2.5 to 15 μg of 5-hydroxytryptamine by intra-arterial injection increased spontaneous activity in the postganglionic fibres from the inferior mesenteric ganglion.

The occurrence of 5-hydroxytryptamine in vertebrate sympathetic ganglia has, as yet, not been demonstrated. However, Gertner, Paasonen & Giarman (1957) observed that the cat superior cervical ganglion is capable of forming 5-hydroxytryptamine when perfused with iproniazid but found that this monoamine oxidase inhibitor produced partial inhibition of transmission through the ganglion. They

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stated that homogenates of sympathetic ganglia possess a higher capacity for catalytic formation of 5-hydroxytryptamine than did any other structure of the nervous system.

The following experiments were performed in an attempt to shed some light on the role of 5-hydroxytryptamine in sympathetic activity.

A preliminary report of this work was made to the meeting of the Federation of American Societies for Experimental Biology in 1960 at Atlantic City, N.J., U.S.A.

METHOD

Preparation of tissue

A portion of the thoracic and cervical regions of the sympathetic trunk was rapidly dissected free from adult Sprague-Dawley rats. The tissue isolated consisted of the stellate ganglion, about 5 mm of the thoracic chain and the entire cervical sympathetic trunk exclusive of the superior cervical ganglion. It was essential that the sheath or capsule surrounding the stellate ganglion be partially or completely removed during the dissection. The preparation was bathed in Krebs solution with a reduced bicarbonate concentration as recommended by Eccles (1952). The solution was equilibrated with 95% oxygen and 5% carbon dioxide to a *p*H of 7.3 to 7.4 at 35 to 36° C.

Apparatus

The preparation was suspended horizontally on platinum electrodes as shown in Fig. 1. The thoracic or preganglionic end was stimulated with rectangular pulses of 0.1 msec duration and an intensity of 0.1 to 15 volts. In experiments on the amplitude of responses, a stimulus intensity was chosen which elicited about one-third of the maximal response. This submaximal stimulus intensity, once chosen, remained unchanged during the experiment.

The threshold of responses was determined by varying the intensity of the preganglionic stimulus to obtain a constant but minimal response.

The problem of maintaining the tissue in a liquid environment but recording in a non-conducting medium was met by automatically lifting the preparation out of solution periodically (Fig. 2). A programme timer controlled a solenoid which, when activated, pulled the electrode assembly downward and hence immersed the ganglion preparation in Krebs or test solution.

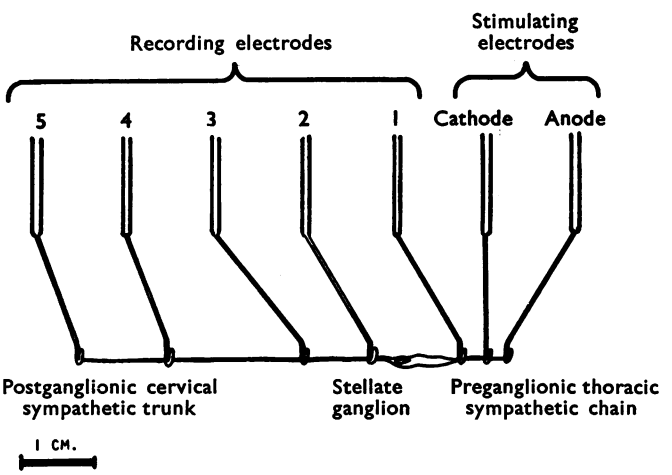

Fig. 1. Arrangement of sympathetic trunk on electrodes. Stimulating pulses were applied through a radio-frequency isolation unit. Recording electrode 1 was earthed and records were obtained from electrodes 1 and 2, or 1 and 3, 4 or 5.

The immersion continued for 30 sec of each minute after which the solenoid circuit was broken and spring tension pulled the electrode carrier out of the solution into the moist air of the chamber. This chamber was virtually sealed and its water jacket held the temperature at 36° C. Stimulation and recording were automatically performed after 25 sec of equilibration in the moist air and 5 sec later the immersion was repeated. This cycle was continued throughout the experiment.

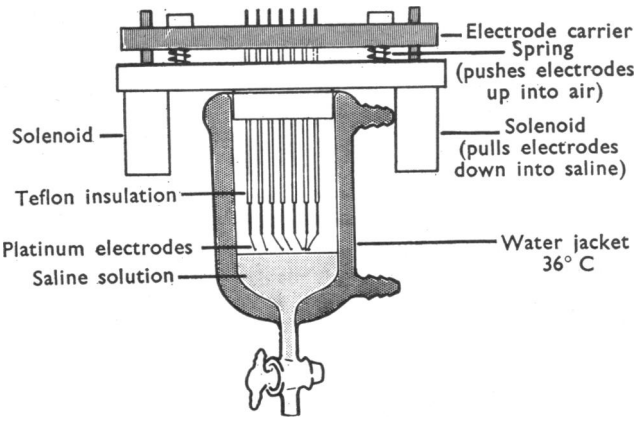

Fig. 2. Details of construction of nerve chamber and electrode assembly. Nerve was periodically immersed in solution and lifted out for recording by the action of a solenoid controlled by a programme timer.

Attempts to perform the sequence of events of immersion, elevation, equilibration with moist air and stimulation manually indicated that timing of the events must be precise if reproducible recordings were to be obtained.

All experiments were performed during the first few hours after dissection although the preparation was responsive for many hours. The potential S_b (Fig. 3) could be elicited for 6 or 8 hr while potential S_a could be elicited for 72 hr.

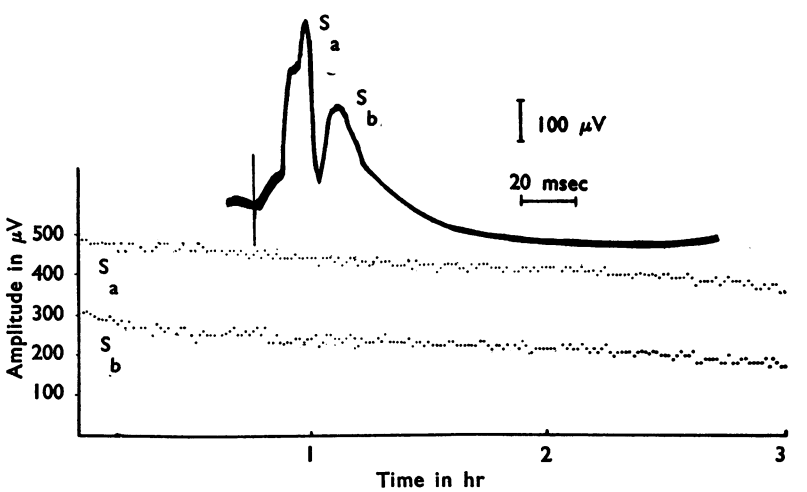

Fig. 3. Above: Response to a submaximal preganglionic stimulus. Records obtained between electrodes 1 and 2 of Fig. 1. Intermittent exposure to control solution only. Below: Scatter diagram of amplitudes of responses (S_a and S_b) at 1 min intervals over a 3-hr period.

Experiments in vivo

Three experiments were performed in which rats under light pentobarbitone anaesthesia were artificially respired. Stimulating electrodes were placed on the thoracic sympathetic trunk and recording electrodes on the ipsilateral cervical sympathetic trunk, thus simulating the experimental conditions used with the isolated ganglion.

RESULTS

Fig. 3 (upper part) illustrates the response obtained from a single submaximal preganglionic stimulus. The artifact is followed by a complex of responses. In conformity with previously accepted terminology, the major components are labelled S_a and S_b. This paper is concerned with the effects of compounds on the amplitudes of these two responses or on the stimulus thresholds necessary to arouse these responses.

Fig. 3 (lower part) gives a scatter diagram of the amplitudes of responses obtained at 1 min intervals for 3 hr during intermittent immersion in Krebs solution. There is shown a slow decline in responses at the rate of about 0.25%/min and a maximum variation or scatter of 10% from an eye fit line of regression.

In preliminary work, 112 experiments were made before the experimental method was developed in its final form. Then 40 measurements were made with various concentrations of 5-hydroxytryptamine. The amplitude of the response was increased by 5-hydroxytryptamine in 36 experiments (range +7.4 to +206%); there was no change in 3 and there was a decrease in 1 (−4%).

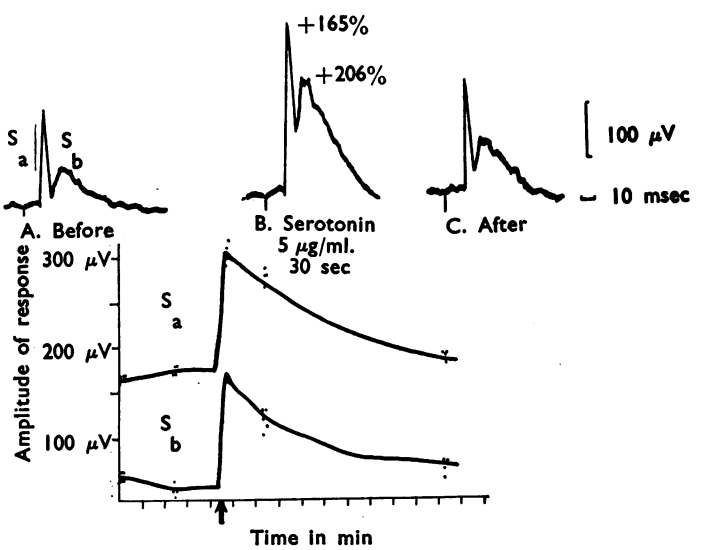

Fig. 4. Above: Responses recorded between electrodes 1 and 2 of Fig. 1. A, Control, response before experiment; B, experimental, response immediately after exposure of preparation to 5-hydroxytryptamine for 30 sec; C, recovery, response after 10 min in control solution (Krebs). Below: Time course of amplitude of S_a and S_b responses. Arrow indicates time of immersion in 5-hydroxytryptamine for 30 sec.

The quantitative variation seen in these results may reflect uncontrolled variables such as injury to the tissue, variable degree of penetration of 5-hydroxytryptamine into the active cells and the impossibility of using comparable submaximal stimuli in successive preparations.

After exposure for 30 sec to 5 μg/ml. (1.3×10^{-5} M) of 5-hydroxytryptamine creatinine sulphate added to the chamber fluid, there was an immediate effect on the response amplitude (Fig. 4). The amplitude of S_a had increased to 165% and that of S_b had increased to 206% of the control responses. Washing in Krebs solution for about 20 min was necessary before the potentials returned to their original values.

TABLE 1

EFFECT OF 5-HYDROXYTRYPTAMINE ON AMPLITUDE ON THE S_a AND S_b RESPONSES

Conc. of 5-hydroxytryptamine (μg/ml.)	Change in amplitude (%)	
	S_a	S_b
0·5	14	31
2·0	20	48
4·0	23	52
8·0	40	86
16·0	40	94
32·0	33	76
64·0	30	27

The amplitude of the response was related to the concentration of 5-hydroxytryptamine. In the experiment in Table 1, the maximum increase in response was produced by a 5-hydroxytryptamine concentration of 16 μg/ml. (4.2×10^{-5} M), while higher concentrations caused less increase.

These results suggested that 5-hydroxytryptamine might be acting by reducing the threshold of preganglionic fibres since submaximal stimuli were used. With a lower threshold, a greater number of fibres would be activated by a given submaximal stimulus and this change in threshold might account for the increased amplitudes of both responses.

Determination of the effect on threshold proved difficult. In most experiments, the threshold of responses to preganglionic stimulation was not sharply limited. However, in some ganglia this threshold was especially critical and the value of minimal stimulus intensity for a constant, small response did not vary during repeated trials. In such a preparation low concentrations of 5-hydroxytryptamine reduced the threshold of responses to preganglionic stimuli whereas high concentrations had the opposite effect (Table 2). In most experiments, S_b response was more sensitive to the addition of 5-hydroxytryptamine than was the S_a response.

TABLE 2

EFFECT OF 5-HYDROXYTRYPTAMINE ON THRESHOLD OF RESPONSES

Conc. of 5-hydroxytryptamine (μg/ml.)	Threshold change (%)
0·1	−5
0·5	−11
5·0	−23
40·0	+18

A series of ganglion blocking agents was next used to find whether they, too, had a differential effect upon the two potentials. Tetraethylammonium, tubocurarine and pempidine all had a greater effect on S_b than on S_a (Table 3).

TABLE 3

EFFECT OF GANGLION BLOCKING AGENTS ON AMPLITUDE OF RESPONSES

		Change in amplitude (%)	
Agent	Conc.	S_a	S_b
TEAC	5 μg/ml.	−10	−36
	10	−25	−75
	250	−100	−100
Tubocurarine	0·3 mg/ml.	Unchanged	Unchanged
	0·6	Unchanged	Unchanged
	0·9	−10	−22
	1·2	−10	−50
	1·5	−20	−55
	3·0	−20	−100
Pempidine	1 μg/ml.	−2	−33
	6	−18	−61
	10	−41	−84

TEAC=Tetraethylammonium chloride (Etamon chloride), Parke-Davis. Tubocurarine=Intocostrin, tubocurarine chloride pentahydrate, Squibb. Pempidine tartrate, May and Baker.

Similar experiments were performed using some "anti-serotonin" substances. Bromolysergic acid diethylamide increased the amplitude of responses in low concentration but decreased them in higher concentration (Table 4). Methysergide (1-methyl-D-lysergic acid butanolamide) had no effect in low concentrations and increased responses at higher concentrations.

TABLE 4

EFFECT OF ANTI-5-HYDROXYTRYPTAMINE SUBSTANCES ON THE AMPLITUDE OF RESPONSES

		Change in amplitude (%)	
Agent	Conc.	S_a	S_b
BOL-148	2 μg/ml.	+28	+17
	5	−22	−33
UML-491	2 μg/ml.	Unchanged	Unchanged
	5	Unchanged	Unchanged
	15	+15	+18
	25	+15	+32

BOL-148=Bromolysergic acid diethylamide bitartrate, Sandoz.
UML-491=Methysergide, Sandoz.

It was of interest to know whether similar results could be obtained in the intact animal. Rats, lightly anaesthetized with pentobarbitone, were artificially respired while the chest was opened and stimulating electrodes were placed on the sympathetic trunk at the level of the third to fourth rib. Polyethylene plastic film was used to prevent the electrodes from coming in contact with nearby tissues and the exposed area was covered with medicinal liquid paraffin. The recording electrodes on the cervical sympathetic trunk were also similarly protected. The position of the stimulating and recording electrodes simulated those in the *in vitro* experiments as closely as possible.

The control postganglionic response to a standard submaximal preganglionic stimulus following the intravenous injection of Krebs solution is shown in Fig. 5 (left-hand response). After a single rapid intravenous injection of 20 μg of 5-hydroxytryptamine, the response was increased by 43% in 30 sec (Fig. 5, middle record). An intravenous dose of 5 mg of tetraethylammonium chloride reduced the response by 76% (Fig. 5, right-hand record). In all such experiments the results were similar.

Fig. 5. Records of postganglionic responses to submaximal preganglionic stimuli in the living rat. Left, control; middle, 30 sec after intravenous injection of 20 μg of 5-hydroxytryptamine (serotonin); right, after intravenous injection of 5 mg of tetraethylammonium chloride.

DISCUSSION

The results suggest that low concentrations of 5-hydroxytryptamine increased the electrical response of the rat stellate ganglion evoked by submaximal preganglionic stimulation.

The action potential seen was a compound response with two spikes similar to those designated as S_a and S_b by Eccles (1952). She concluded that both of the components were ganglionic (postsynaptic) in origin; that the ". . . ganglionic S_a spike discharge begins so soon after the arrival of the fast preganglionic spike that it obscures most of the diphasic preganglionic spike . . ." The activity of non-synaptic fibres (those passing through the stellate ganglion to synapse in the superior cervical ganglion) must likewise be masked in the S_a wave form.

Shaw, MacCullum, Dewhurst & Mainland (1951) have also advanced evidence to support the idea that drugs can act differently upon the S_a and S_b response, and contend that there may be two pharmacologically distinct groups of cells in sympathetic ganglia. It is possible that the S_a and S_b components of the action potential in these experiments also represent the activity of two such groups of cells, 5-hydroxytryptamine having a more pronounced effect on the cells responsible for the S_b discharge.

Woolley (1958) has suggested that 5-hydroxytryptamine acts by carrying calcium ions into the uterine muscle cell and thus activates the actomyosin-adenosine triphosphate system. A few experiments were performed in which the technique of Woolley (1958) was adapted to the study of sympathetic ganglia and the results were inconclusive. It did not appear that calcium transport is dependent on 5-hydroxytryptamine in ganglia.

The recent paper by Bülbring & Burnstock (1960) shows that 5-hydroxytryptamine produces a partial depolarization of smooth muscle fibres. If this should also be true for sympathetic nerve fibres, then it would provide an explanation of the changes observed.

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