Evidence for Serotonin (5-Hydroxytryptamine) as Transmitter in the Penis Retractor Muscle of *Helix pomatia* L.

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Summary. Microchromatography of dansylated substances was used to estimate the serotonin content of the penis retractor muscle (PRM) of *Helix pomatia*. Pharmacological data in connection with the biochemical results suggest that 5-HT plays a role in the regulation of the mechanical activity of the PRM.

The significance of 5-hydroxytryptamine (5-Ht) as a physiologically active substance in certain molluscan nerve-nerve and nerve-muscle synapses is generally accepted (Florey[1], Gerschenfeld[2]). 5-Ht, together with enzymes for its synthesis and its breakdown, have been shown to occur also in *Helix* central nervous system and neuromuscular junction (Kerkut et al.[3], Sedden et al.[4], Juorio et al.[5], Osborne et al.[6]). However, the question of whether 5-Ht may act as neuromuscular transmitter within the penis retractor muscle (PRM) of *Helix pomatia* has not been answered. Using a fluorescence histochemical technique, Bogusch[7] demonstrated a yellowish formaldehyde-induced fluorescence (indicative of 5-Ht) within the varicosities of neuronal elements in the PRM. An extensive biochemical analysis of intramuscular 5-Ht, as well as a pharmacological test of the effect of the biogenic amine on the PRM, is still lacking. The present experiments were designed firstly to study the action of 5-Ht on the isolated PRM preparation. The second purpose of the present study was to determine the concentration of 5-Ht in the PRM by the sensitive dansylation method (Neuhoff[8]). Thirdly, the presence of free amino acids in this muscle was examined with regard to further investigations of their possible metabolic functions and/or their role in the neuromuscular transmission.

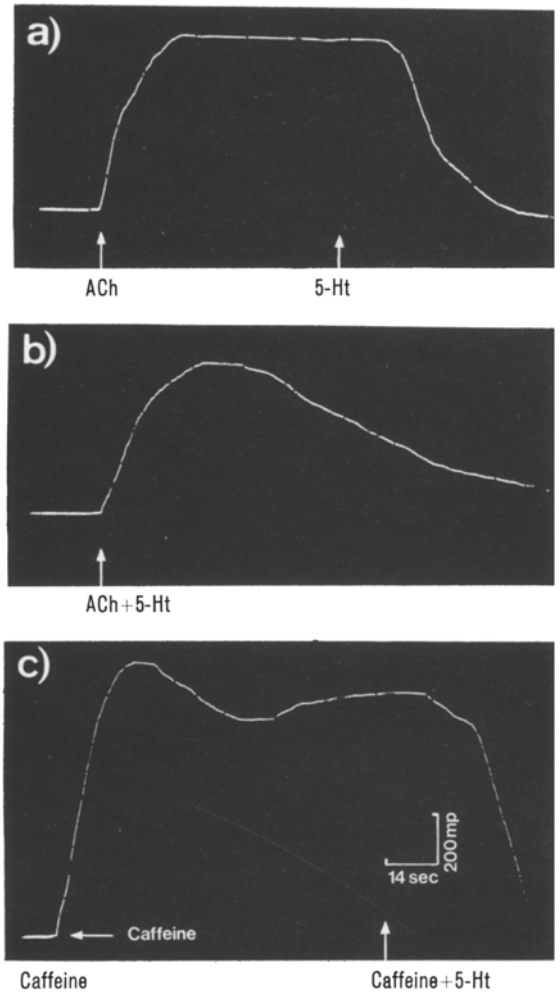

Fig. 1. Tension developed by an isolated PRM preparation. The first contraction (a) was induced by ACh injection into the normal saline to final concentration of 5×10^{-4} *M*. The application of 5-HT (10^{-7} *M*) in addition to ACh caused relaxation of the PRM. Preexposure in 5-HT (10^{-7} *M*,) for 10 min caused a transient ACh contraction (b). The administration of caffeine (10^{-2} *M*) caused a double contraction (c). The second slow component could be relaxed by 5-HT (10^{-7} *M*).

Materials and methods. Specimens of *Helix pomatia* L. weighing 20–30 g including the shells were used. The experiments were performed on the isolated penis retractor muscles of active snails. The preparation has been described in detail by Wabnitz[9]. The pharmacological experiments were carried out after an equilibration period of 60 min in oxygenated ringer solution: NaCl 51.0 m*M*/l, KCl 3.7 m*M*/l, $CaCl_2$ 10.0 m*M*/l, $MgCl_2$ 12.6 m*M*/l, glucose 1.0 m*M*/l, *Tris* 5.0 m*M*/l. The pH was adjusted to 7.2 by the addition of NaOH.

Auxotonic contractile force recordings were obtained by using a force transducer. The signals wer photographed from a Tektronix Type 5 103 N Dual-Beam Storage oscilloscope.

To analyze 5-Ht and free amino acids, the freshly dissected tissue was homogenized in sodium carbonate buffer, pH 10.0 (1 mg per 100 µl) and centrifuged at $22{,}000 \times g$ at 0 °C for 30 min. To the supernatant twice the volume of acetone was added, vigorously shaken and stored in a refrigerator at −28 °C for 1 h. The precipitated proteins were removed by centrifugation (30 min, $22{,}000 \times g$, 0 °C); 4 µl of the clear supernatant were transferred to a clean microglass tube and reacted with an equal volume of ^{14}C-dansyl-chloride (Amersham-Buchler, Braunschweig; specific activity 20.3 mCi/mmol; concentration 3 mg/ml acetone) for 30 min at 37 °C in the dark. The solvent was evaporated and the residue dissolved in 5 µl actone/acetic

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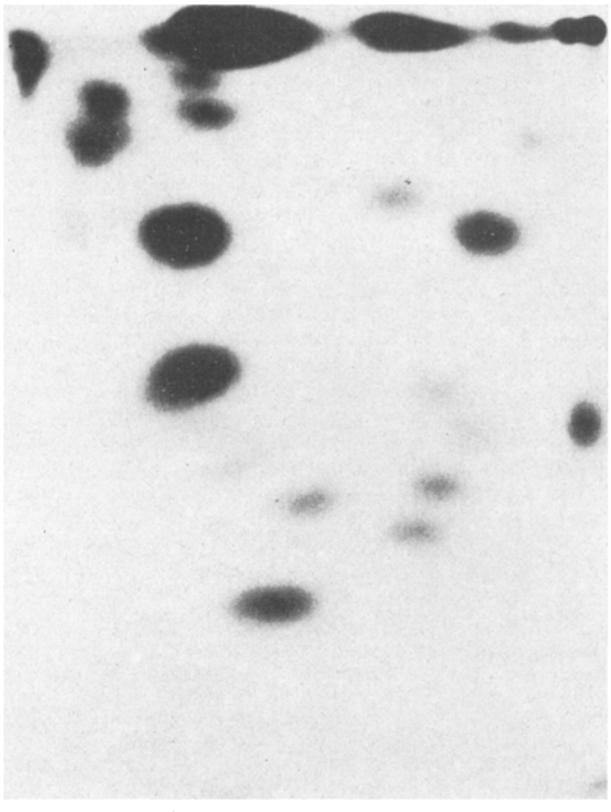
Composition of substances separated by microchromatography

Spot No.	Dansylated substance occurrence	
1	Taurine	+++
2	^{14}C-Dansyl-Chloride-impurities	
3	Arginin and α-amino-histidin	++
4	*N*-Serotonin	+
5	Glutamic acid	++
6	Aspartic acid	++
7	Glutamine	++
8	Serine	++
9	Asparagine	+
10	Threonine (as dansyl-OH)	+
11	Tryptophan	+
12	*bis*-Lysine	+++
13	Ornithine	+
14	Glycine	+++
15	Unknown	+
16	Alanine (as dansyl-OH)	+++
17	GABA	+
18	Phenylalanine	+
19	*bis*-Histidine	+
20	Leucine	+
21	Isoleucine	+
22	*bis*-Tyrosine	++
23	*bis*-Serotonin	+
24	Valine	+
25	Proline	+++
26	Unknown	+
27	Unknown	+
28	5-Hydroxyindole	+
29	Unknown	+
30	^{14}C-Dansylchloride impurities	
31	see No. 30	

acid (3:2, v/v). Aliquots of 0.2 µl were spotted onto a corner of a 3×3 cm polyamide layer (F 1700 micropolyamide sheet, Schleicher & Schüll) and developed by two dimensional chromatography (Figure 2a). The fluorescent spots obtained were marked with a soft pencil under UV-light (254 nm), scraped off with a special knife (NEUHOFF[8]) and immediately transferred into vials, which contained 10 ml of a scintillation liquid (4 g PPO and 0.1 g POPOP in 1 l toluene for scintillation counting) and counted in a Packard liquid scintillation counting spectrometer, model 2425. Results have been obtained for 8 experiments; after a correction for quenching, the resulting counts could be converted into µmoles of 5-Ht using a calibration curve for dansylated 5-Ht.

Results and discussion. It has been shown by mechanical and electromyographical studies that in the intact penis retractor muscle-nerve-brain preparation 2 distinct types of neurogenic elicited contractions exist: a) a phasic contraction and b) a tonic contraction (WABNITZ[9]). With ACh and 5-Ht it was possible to mimic contractions in the isolated PRM preparation (free of peripheral ganglion cells) similar to those occurring spontaneously in the intact preparation.

Figures 1a and b show the excitatory effect of ACh and the relaxing action of 5-Ht. The effective concentration of ACh was $\geq 10^{-5}$ *M* and that of 5-Ht $\geq 10^{-7}$ *M*. While ACh caused a tonic contraction followed only by a slow decrease of the tonus, the application of both ACh and 5-Ht induced a transient contraction. Studies of the effect of facilatory and inhibitory agents of cholinergic transmission on the PRM (WABNITZ[10]) as well as the demonstration of ACh-esterase located on the sarcolemma of



2a

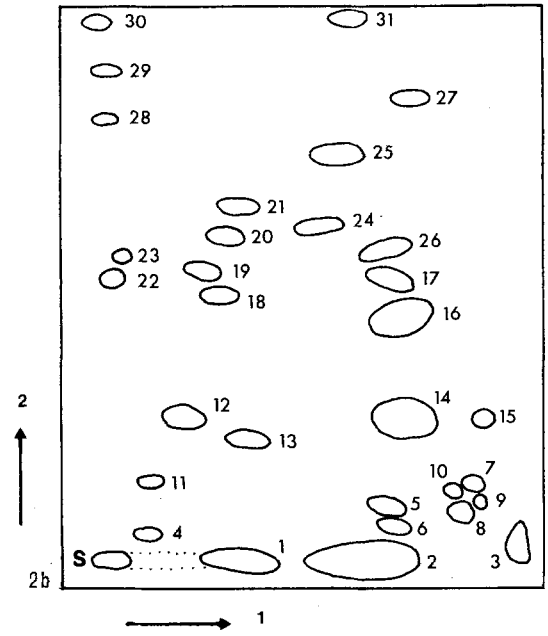


Fig. 2. The figure shows a) the autoradiogram of the microchromatogram from the extract of the penis retractor muscle and b) the corresponding map; the numbers of the map correspond to the dansyl-compounds as shown in the Table. The direction of chromatography is indicated by arrows, first direction: water-formic acid (100:3 v/v), second direction: benzene-acetic acid (9:1 v/v), S: Starting point. Exposition time of the autoradiograms 7 days.

PRM muscle cells (BOGUSCH[11]) indicate that ACh may be the excitatory transmitter in the PRM. On the other hand, our investigations have shown that the relaxing effect of 5-Ht could be potentiated by the administration of Lilly 110 140 (5×10^{-4} *M*) a selective inhibitor of 5-Ht uptake (WONG[12]). According to BENNETT[13], one mechanism involved in inactivating biogenic amines appears to be the re-uptake into the nerve ending which has released them. High affinity uptake of biogenic amines have also been reported to exist in glial cells.

5-Ht and ACh are also implicated in the regulation of phasic and tonic contractions in the molluscan smooth muscle preparation of the anterior byssus retractor muscle (ABRM) of *Mytilus edulis* (TWAROG[14], YORK[15]). MARCHAND-DUMONT et al.[16] conclude from their experiments on the ABRM that the muscular membrane is necessary for the relaxing effects of 5-Ht and that these effects are mediated through cyclic AMP. The relaxing mechanism of the PRM seems also to be mediated by this cyclic nucleotide, functioning as intracellular messenger. Figure 1c shows the response of the curarized (10^{-3} *M*) PRM preparation to caffeine (10^{-2}) which is known to release calcium from intracellular storage sites to activate the contractile apparatus (WABNITZ[17]). The caffeine contraction could be relaxed by 5-HT (Figure 1c) and also by dibutyryl cyclic AMP (WABNITZ et al. in preparation) indicating that 1. 5-Ht may play a part in the intracellular calcium regulation and /or 2. 5-Ht is able to release calcium from the contractile proteins (MARCHAND-DUMONT et al.[16]).

Figures 2a, b and the Table show the results of a representative analysis of the presence of 5-Ht and free amino acids in the PRM obtained by the sensitive dansylation method (NEUHOFF[8]). From the autoradiogram the occurrence of 29 substances which have reacted with ^{14}C-dansylchloride in the extract of the homogenate of a resting PRM can be seen. 5-Ht, which occurs as dansyl-*N*-serotonin (Figure 2, No. 4) and as dansyl-*bis*-serotonin (Figure 2, No. 23) could definitely be proved. By measuring the radioactivity of these two substances, the content of free 5-Ht within the PRM could be determined. The amount of measured 5-Ht in electrically and pharmacologically untreated muscles was 3.3 $\pm$ 0.3 µg/g wet tissue. Electrical stimulation (square pulses, 0.4 msec, 3V, 0.1 Hz, 30 min) applied to the isolated PRM preparation perfused with ringer solution containing the 5-Ht re-uptake inhibitor Lilly 110 140 produced a decay of the 5-Ht content. The amount of measured 5-Ht after electrical and pharmacological treatment was 2.1 $\pm$ 0.2 µg/g wet tissue. From our pharmacological and biochemical data and the histochemical observations by BOGUSCH[7], we can be relatively certain that 5-Ht plays a role in the regulation of the mechanical activity of the PRM in vivo.

The following amino acids, whose role in other tissues as transmitter substances will be discussed (for review see GERSCHENFELD[2]), are also found in the PRM: aspartate, γ-aminobutyrate, glutamate, glycine and taurine. Whether any of these compounds serve as neurotransmitter or are involved in intracellular mechanisms of 'catch' muscles is not yet known, but their role is clearly worthy of investigation (VON WACHTENDONK et al., in preparation).

Furthermore, the appearance of 3 new unidentified substances seems to us to be of some interest because these substances were found to be in the PRM of *Helix pomatia*, as well as in the ABRM of *Mytilus* (KÄPPLER et al.[18]) and their presence is possibly limited to 'catch' muscles.

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Intestinal Absorption of Glucose Immediately after Vincristin Administration in Rats

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Summary. Vincristin leads to a time-dependent decrease of glucose absorption. Thus it is not possible to combine experiments which seek simultaneous information on intestinal absorption and epithelial replacement.

GLICKMAN[2] could show that colchicine, a substance which influences the microtubular system, diminished fat absorption as early as 2 h after administration by influencing solely the extrusion of the chylomicrons. The present study was performed to investigate whether or not measurement of in vivo absorption of water-soluble substrates (glucose, Na^+ and K^+) can be combined with measurement of cell production according to CLARKE[3].

Methods. 17 male outbred rats of a Wistar strain (SV 49 Thomae, Biberach) with an average body weight of 200 g were kept under SPF conditions in a Trexler plastic isolator in wire bottom cages. On the day prior to investigation, solid food (Altromin 14/15 fortified, Altrogge, Lage Lippe) was withheld at the beginning of the dark period (19.00 to 07.00 h) with free access to water. After i.p. pentobarbital anesthesia, the rats received an i.v. injection at 09.00 h of vincristin (1 mg/kg body weight; $n = 8$) or an equal amount of Ringer solution (3 ml/kg body weight; $n = 9$). After this a 15 cm jejunal segment just distal to the ligamentum of Treitz was cannulated

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