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Receptors of neurotransmitters—III. Comparison of the 5-hydroxytryptamine receptor of the liver fluke, *Fasciola hepatica*, and the rat stomach-fundus

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EARLIER investigations on smooth muscle indicated a relationship between the receptor function for 5-HT* and sialic acid metabolism.[1] Different *N*-acyl-neuraminic acids and gangliosides increased sensitivity as well as maximal height of 5-HT induced contraction of the rat stomach fundus while synthetic inhibitors of NANA-biosynthesis decreased the contraction. In an attempt to get further information about the 5-HT receptor by comparing receptors from different species, we studied the influence of sialic acid metabolism and of adamantanamines on the 5-HT induced activity of the liver fluke, *Fasciola hepatica*. The adamantanamines were found to give an increased response of the rat fundus strip to 5-HT[2].

According to Welsh[3] 5-HT may play a role as mediator in nerve action in certain invertebrates. Erspamer[4] found 5-HT and other biological active indolamines in many tissues of invertebrates.

Liver flukes were collected from bovine livers within 1 hr after death of the host and kept in buffered (dH 8·5) Ringer's solution at 37° as described by Chance and Mansour.[5] Kymograph registrograms were made within 24 hr after dissecting the flukes. The fluke was incubated in 10 ml Ringer's solution at 37°. Oxygen was passed through the vessel to facilitate the distribution of added compounds. The fluke being under slight tension[5] was fixed to one end of an isotonic level giving about 5 times magnification and allowed 10 min to acclimatize before starting with experiments. Each contraction was registered for 5 min and the drug was washed out for 10 min before the following contraction was produced. Thus one preparation could be used for 90–120 min.

Using 5×10^{-4} M 5-HT or amphetamine sulphate as an agonist the contraction height of the fluke can be maintained constant for at least 90 min (Fig. 1). Mansour[6] suggested that amphetamine and

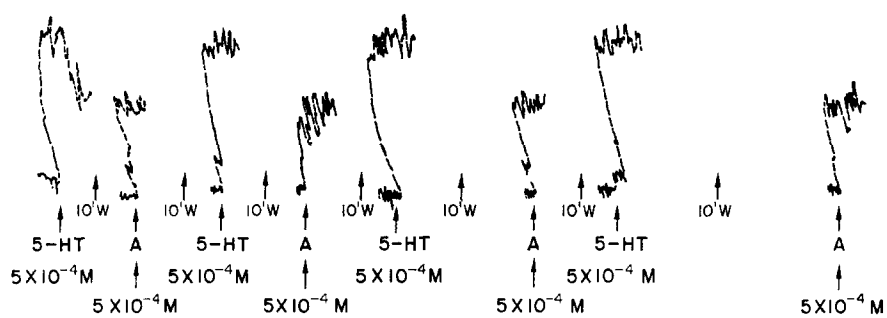


FIG. 1. Kymograph registrogram of 5-HT and amphetamine sulphate (A) induced contraction on the liver fluke, concentration 5×10^{-4} M.

* Abbreviations used: 5-HT = 5-hydroxytryptamine (serotonin); NANA = *N*-acetylneuraminic acid; NANA-9-P = *N*-acetylneuraminic acid-9- phosphate.

5-HT may act on the same receptor. Incubation of the liver fluke with a 10^{-4} M solution of NANA for 15 min results in a gradual increase of the 5-HT contraction (Fig. 2). After washing out the NANA, a 5-HT contraction was produced every 15 min, recorded over a period of 5 min and followed by a 10 min wash procedure. The maximal effect was obtained 40 min after incubation with NANA.

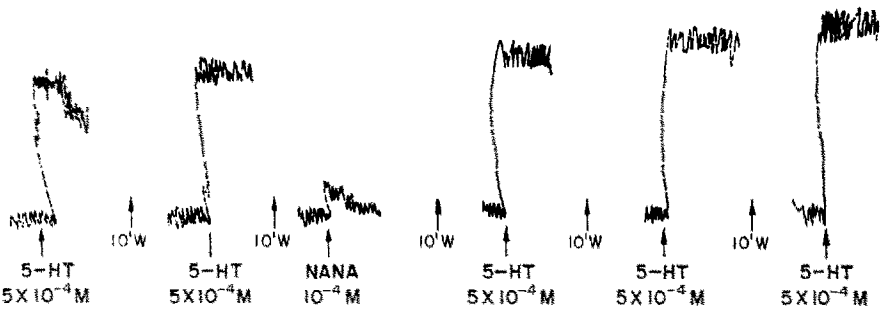


FIG. 2. Effect of 15 min preincubation with a 10^{-4} M solution of NANA on the 5-HT contraction of the liver fluke.

From the three *N*-acylneuraminic acids tested (Table 1), the most lipophilic substituted *N*-benzoyl-derivative yields the highest increase in 5-HT contraction.This agrees with the results previously reported with the fundus strip preparation[1]. The increase in contraction after incubation of the fluke with a mixture of gangliosides is about 10 per cent less as compared with the rat stomach fundus.

TABLE 1. INFLUENCE OF 15 MIN PREINCUBATION WITH SIALIC ACIDS AND GANGLIOSIDES ON THE 5-HT CONTRACTION OF THE LIVER FLUKE

Compound*	Contraction height (%)† 10 min after incubation	40 min after incubation	No. preparations
N-acetyl-NA	110–115	130–135	6
N-cbo-NA	110–115	130–140	5
N-benzoyl-NA	120–125	145–150	4
Gangliosides‡ (mixture)	110–115	115–120	4

* Each compound was tested in a concentration of 10^{-4} M.

† The 5-HT contraction was produced with a 5×10^{-4} M solution. The 5-HT contraction of the untreated preparation was set 100 per cent,

‡ Purified beefbrain gangliosides Type II from Sigma Chemical Company.

Synthetic *bis*-(2,4,5-trichlorphenoxy)-acetic acid specifically inhibits the enzyme NANA-9-P-synthetase and decreases reversibly the 5-HT contraction of the rat stomach fundus. NANA-9-P-synthetase catalyzes the synthesis of NANA-9-P from *N*-acetylmannosamine-6-P and phosphoenol-pyruvate. After preincubation of the liver fluke with $1{\cdot}7 \times 10^{-5}$ M *bis* (2,4,5-trichlorphenoxy)-acetic acid for 15 min followed by the wash procedure for 10 min, no contraction could be produced with 5×10^{-4} M 5-HT. Forty minutes after preincubation about 80 per cent of the contraction height of the untreated preparation could be obtained. Attempts to get a 100 per cent recovery of the fluke failed. *N*-salicylidene-D-glucosamine and *N*-[D,L-α-propionic acid-ethylester-salicylidene]-D-glucosamine—other potent inhibitors of NANA-biosynthesis[7] and 5-HT induced contraction of rat fundus[1]—decrease in rather high concentrations (10^{-3} M) the contraction of the liver fluke (Table 2).

The antiviral compound adamantan-1-amine and some of its derivatives were found to sensitize the rat fundus preparation towards 5-HT without changing the contraction height[8]. The sensitization was expressed as a factor by which the 5-HT log dose-response curve was shifted towards lower

TABLE 2. INFLUENCE OF 15 MIN PREINCUBATION WITH INHIBITORS OF NANA-BIOSYNTHESIS ON THE 5-HT CONTRACTION OF THE LIVER FLUKE

Compound	conc. (M)	Contraction height* (%) 10 min after incubation	25 min after incubation	40 min after incubation	No. preparations
Bis-(2,4,5-trichlorphenoxy)-acetic acid† sodium salt	$1{\cdot}7 \times 10^{-5}$	0	50–55	75–80	5
N-salicylidene-D-glucosamine†	10^{-3}	45–55	75–85	85–90	4
N-(D,L-α-propionic acid-ethyl-ester-salicylidene)-D-glucosamine†	10^{-3}	30–35	70–80	75–80	4

* The 5-HT contraction was produced with a 5×10^{-4} M solution. The 5-HT contraction of the untreated preparation was set 100 per cent.

† Th. A. C. Boschmann, in press.

concentrations. A true 5-HT log dose-response curve of the liver fluke could not be obtained because the maximal 5-HT produced contraction of the liver fluke is small compared with the rat fundus preparation and the relative high amplitude of rhythmical movement is further increased by 5-HT. In order to get significant differences in contraction height, only two different 5-HT concentrations,

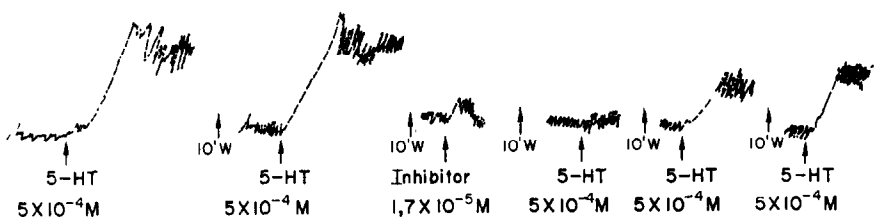


FIG. 3. Influence of 15 min preincubation with a $1{\cdot}7 \times 10^{-5}$ M solution of *bis*-(2,4,5-trichlor-phenoxy)-acetic acid on the 5-HT contraction of the liver fluke.

5×10^{-5} M and 5×10^{-4}M, were used. The first one was just above the minimum effective concentration, the latter one produced in most preparations tested the maximal contraction height which can be obtained with 5-HT.

With a concentration of 5×10^{-5} M HT about half maximal contraction height is obtained (Fig. 4). The contraction increases to about 90 and 100 per cent of the maximal value in the presence of

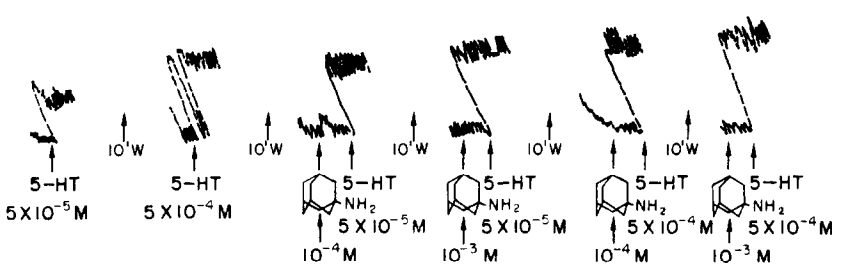
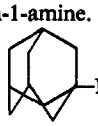


FIG. 4. Effect of adamantan-1-amine. HCl on the 5-HT contraction obtained on the liver fluke.



= adamantan-1-amine.HCl

adamantan-1-amine in a concentration of 10^{-4} M and 10^{-3} M respectively. The same two concentrations of adamantan-1-amine give only a relative small increase (maximum 15 per cent) in contraction height if a 5×10^{-4} M solution of 5-HT is used. The results obtained with adamantan-1-amine and its *N*-ethyl-derivative (Table 3) suggest that these compounds do not increase the intrinsic activity

TABLE 3. EFFECT OF ADAMANTANAMINES ON THE 5-HT INDUCED CONTRACTION OF THE LIVER FLUKE

conc. 5-HT (M)	compound added	conc. (M)	contraction height* (%)	No. curves
5×10^{-4}	none	—	100	—
	Adamantan-1-amine. HCl	10^{-4}	105–110	5
		10^{-3}	110–115	4
	N-Ethyladamantan-1-amine.HBr	10^{-4}	100–105	4
		10^{-3}	110–115	4
	N-(Adamant-1-*yl*-)urea	10^{-7}	95–105	3
		10^{-6}	105–110	4
5×10^{-5}	none	—	50–60	25
	Adamantan-1-amine.HCl	10^{-4}	85–90	8
		10^{-3}	100–105	8
	N-Ethyladamantan-1-amine.HBr	10^{-4}	80– 85	6
		10^{-3}	95–100	6
	N-Adamant-1-*yl*)urea	10^{-7}	55–60	10
		10^{-6}	55–65	10

* The 5-HT contraction of the untreated preparation obtained with a 5×10^{-4} M solution was set 100 per cent.

since they do not increase the maximal height significantly. They seem to be rather sensitizers of the 5-HT induced contraction of the liver fluke. This agrees with the results obtained on the rat fundus strip preparation. The lack of any sensitizing effect of *N*-(adamant-1-yl)urea which is one of the most potent sensitizers on the rat stomach, cannot as yet be explained.

The experiments described above suggest evidence that the 5-HT receptor of the rat fundus strip and of the liver fluke, *Fasciola hepatica*, have some features in common. Both preparations are sensitized by adamantan-1-amines and in both exists a relationship between 5-HT induced contraction and sialic acid metabolism.

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