The role of sialic acid in 5-HT binding to synaptic membranes[1]

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Summary. The high affinity binding of [^{14}C]5-HT to nerve ending membranes isolated from rat brain is not affected by neuraminidase treatment. The specificity of ligand receptor interaction was demonstrated by displacement studies with tryptamine derivatives, noradrenaline, and acetylcholine.

Nerve ending membranes are rich in glycolipids and glycoproteins with terminal sialic acid residues[2,3]. Sialic acid-containing structures are involved in high affinity 5-HT uptake by isolated nerve endings[4]. In the present report, the role of sialic acid in high affinity 5-HT binding to synaptic membranes was investigated. This study was encouraged by previous findings showing that gangliosides or glycoproteins are related to 5-HT binding or transport[5-7].

Nerve ending membranes were isolated from rat brain after dissecting away cerebellum and medulla oblongata as described previously[8]. The purity of the preparation was tested enzymatically[9] and by electron microscopy. In binding studies, the membrane suspension (protein concentration 0.75-1.00 mg/ml) in KREBS-phosphate buffer, pH 7.2, was equilibrated to 37 °C for 5 min[4]. Thereafter 500 µl aliquots were incubated at 37 °C for 10 min with [^{14}C]5-HT (5-hydroxy [side chain-2-^{14}C] tryptamine creatinine sulfate, 58 mCi/mmole) at a final concentration range from 5 to 100 nM in the absence (total binding) and presence of a 1000-fold excess of unlabeled 5-HT (nonspecific binding). Specific binding was calculated as the difference of nonspecific from total binding. The effect of neuraminidase treatment on 5-HT binding was studied after 20 min preincubation with *Clostridium perfringens* or *Vibrio cholerae* neuraminidase followed by 10 min incubation with [^{14}C]5-HT as described above. The enzyme preparations were free of proteolytic activity and did not bind 5-HT. Total sialic acid was analyzed[10] with 0.1 N H_2SO_4, 80 °C, 60 min. Protein was assayed using bovine serum albumin as standard[11]. Membrane-bound [^{14}C]5-HT was separated from free by filtration (Whatman filters). After treatment with 1 ml 10% (w/v) sodium dodecylsulfate, 22 °C, 14 h, radioactivity of the samples was counted in 15 ml of Bray's scintillation cocktail[12] by means of a Packard Tricarb Liquid Scintillation Spectrometer. Disc electrophoresis[13] of the SDS membrane extracts was performed in 0.025 M phosphate buffer, pH 6.6, 0.1% (w/v) SDS with 5, 7.5, and 10% acrylamide gels at 4 mA/gel. The gels were stained for proteins (Coomassie brillant blue R-250), glycoproteins[14], and sialic acid[15].

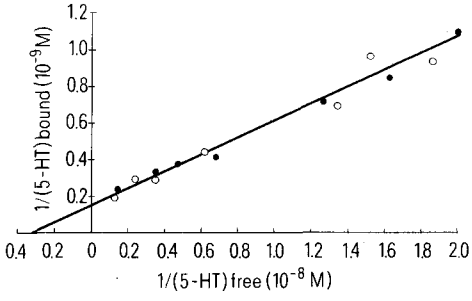
The kinetics of specific [^{14}C]5-HT binding to the membranes exhibited saturation kinetics and a high affinity, $K_D = 30$ nM. Half maximal displacement of specifically bound [^{14}C]5-HT was achieved with 40 nM solutions of unlabelled 5-HT. The number of binding sites was found to be 4.8 pmoles/mg protein. On the premise that the isolated membrane fraction contains only 1 type of 5-HT receptors with 1 class of binding sites, the number of 5-HT receptors can be estimated. Regarding the unknown loss of receptors during purification, the following computation is of speculative nature but nevertheless it may be helpful with respect to receptor isolation and characterization. As 1 g brain wet wt corresponds to 1.2 mg protein of highly purified membranes, the concentration of binding sites per g wet wt is 5.8 pmoles or 3.5×10^{12} receptors. Assuming a mol. wt of 60,000, a 5-HT receptor concentration below 0.4 µg protein per g brain wet wt is expected (calculated 0.35 µg protein/g brain wet wt). Rat brain contains about 10^7 neurons[16] with only 0.067% or 6.7×10^3 of the serotonergic type[17]. On the basis of an average brain weight of 1.8 g about 0.9×10^9 receptors are found per 5-HT neuron.

The specificity of ligand receptor interaction was investigated by competition experiments (table). N-Methylserotonin was most effective in displacing 5-HT, followed by other tryptamine derivatives. Acetylcholine, lacking structural similarity, was without effect and noradrenaline, which was suggested to exhibit some affinity to 5-HT binding sites[18], displaced 25% at a 1000-fold excess. After incubation with neuraminidase, the 5-HT binding sites exhibited high stability (figure). Even a loss of 54.8% of the total membranebound sialic acid (22.6 ± 1.3 nmoles/mg protein) was not paralleled by a decrease in receptor affinity or capacity. In contrast to the distribution of numerous proteins on disc gels, sialic acid was concentrated in few bands (mol. wt 18,200, 19,500, 22,660, 226,800). Though extensive studies on the influence of factors like pH and ions on 5-HT binding to synaptic membranes are still lacking, these findings suggest, with regard to 5-HT receptor isolation, that 1. on a protein basis only a small yield of 5-HT receptors can be expected, and 2. it is not advisable to use isolation procedures based on the binding of sialic acid residues.

Inhibition of specific 5-HT binding by structural analogues

	IC_{50} (M)
N-Methylserotonin	1.5×10^{-7}
Bufotenine	1.3×10^{-6}
Tryptamine	2.2×10^{-6}
5-Methoxytryptamine	7.4×10^{-6}
Cyproheptadine	1.8×10^{-5}

The membranes were incubated at 37 °C for 10 min with 30 nM [^{14}C] 5-HT with and without 30 µM unlabeled 5-HT or different concentrations of competing ligands. The values represent quadruplicate assays with less than 15% variation.



Effect of neuraminidase treatment on specific 5-HT binding. Membranes were incubated at 37 °C, 10 min, with increasing concentrations of [^{14}C]5-HT after 20 min preincubation at 37 °C, in the presence (○——○) and absence (●——●) of *Vibrio cholerae neuraminidase (75* µg/mg membrane protein[19]. All values were corrected for nonspecific binding as described in the text. The points are means of triplicates (SD ± below 10%).

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2 J. Eichberg, V.P. Whittaker and R.M.C. Dawson, Biochem. J. *92,* 91 (1964).
3 H. De Kirmenjian and E.G. Brunngraber. Biochim. biophys. Acta *177,* 1 (1969).
4 G.A. Dette and W. Wesemann, Hoppe-Seyler's Z. Physiol. Chem. *359,* 399 (1978).
5 D.W. Woolley and B.W. Gommi, Nature *202,* 1074 (1964).
6 W.E. Van Heyningen, Nature *249,* 415 (1974).
7 J. Offermeier and E.J. Ariëns, Archs int. Pharmacodyn. *164,* 192 (1966).
8 W. Wesemann, FEBS Lett. *3,* 80 (1969).
9 B. Kadenbach, Biochem. Z. *344,* 49 (1966).
10 D. Aminoff, Biochem. J. *81,* 384 (1961).
11 O.H. Lowry, N.J. Rosebrough, A.L. Farr and R.J. Randall, J. biol. Chem. *193,* 265 (1951).
12 G.A. Bray, Analyt. Biochem. *1,* 279 (1960).
13 K. Weber and M. Osborn, J. biol. Chem. *244,* 4406 (1969).
14 H. Gloosmann and D.M. Neville, J. biol. Chem. *246,* 6339 (1971).
15 E. Heuser, K. Lipp and H. Wiegandt, Analyt. Biochem. *60,* 382 (1974).
16 H. Waelsch, in: Ultrastructure and cellular chemistry of normal tissue. Harper and Row, New York 1962.
17 M.J. Kuhar, Life Sci. *13,* 1623 (1973).
18 E.G. Shaskan and S.H. Snyder, J. Pharmac. exp. Ther. *175,* 404 (1970).
19 E. Mohr and G. Schramm, Z. Naturforsch. *15b,* 568 (1960).

Detection and isolation of induced chromosome aberrations in Lepidoptera

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Summary. An embryo chromosome technique combined with larval testes sampling permits isolation within 1 generation of lepidopteran strains carrying chromosome aberrations.

Identification and isolation of chromosomally aberrant strains of the spruce budworm, *Choristoneura fumiferana* (Clem.) (Lepidoptera: Tortricidae), by conventional genetic techniques are complicated by 2 factors. Firstly, fertilities of single pair matings in the laboratory normally range from 40 to 95%, making detection of translocation carriers by reduction in egg hatch uncertain. Secondly, an obligatory 20-week 2nd instar larval diapause limits the number of generations reared per year to two. An embryo chromosome technique used in conjunction with larval testis sampling reduces the number of generations required to isolate strains carrying particular types of chromosome aberrations from 3 to 1 and minimizes the effort involved in rearing.

Materials and methods. Spruce budworm obtained from stocks maintained at this Institute were reared and mated as previously described[1]. Chromosome counts were obtained from embryos in the following manner. Samples of 24-h-old eggs were gently teased from egg masses and agitated in 1% NaOH at 35 °C for 10 min to separate the eggs[2]. After a brief rinse in modified Ringer-Castillo[3] without Ficoll, eggs were transferred individually to 0.3 ml of 0.02% colchicine in Ringer-Castillo in plastic chambers of a Shandon Cyto-Centrifuge (Shandon Southern Products Ltd, Runcorn, Great Britain). After 20 min at 22 °C, 0.9 ml of sterile distilled water was added to each chamber, eggs were carefully torn open, and the chamber contents gently mixed. After 5 min, cells were centrifuged onto glass slides at 900 rpm for 10 min. Slides were subsequently air-dried for 30 sec, post-fixed for 3 min in ethanol-acetone (1:1) at −20 °C, air-dried, stained with 2% acetocarmine and examined under phase contrast. Usable chromosome preparations were routinely obtained from 9 to 10 of the 12 embryos that could be processed at one time.

Testes were sampled from 5th instar larvae[4]. Survival of surgically-treated larvae to adulthood averaged 77%.

In establishing the utility and reliability of the method, 1-day-old adult males were exposed in groups of 10 to 0, 2 or 10 kR of X-irradiation (400 R/min, Faxotron 804, Field Emission Corp., Oregon) and individually mated to normal virgin females. Egg masses were collected daily and at least 9 embryos/mating were examined for inherited chromosome aberrations.

Results and discussion. Chromosome aberrations were detected in at least some embryos from all 6 successful 2 kR matings. This induced chromosome damage was not reflected by reduction in egg hatch compared to controls. Among embryos examined from the 5 successful 10 kR matings, 71% carried detectable aberrations. Average egg hatch in these families was 52% of the controls. No aberrant embryos were found in the 7 control families.

Aberrations detected were limited to numerical changes, as the small size and large number (2n = 60) of *C. fumiferana*

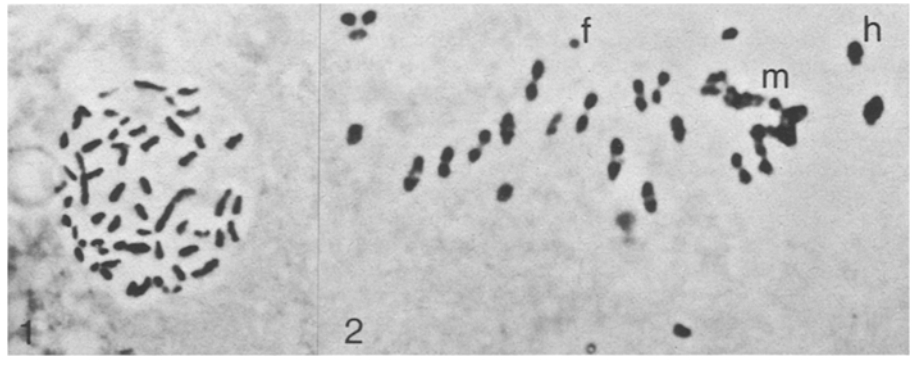

Fig. 1. Inherited chromosome aberrations in *C. fumiferana* embryo that reduced chromosome number from 2n = 60 to 2n = 58. × 1570.
Fig. 2. Meiotic metaphase from male offspring of an adult male irradiated with 10 kR. Note presence of fragment (f), heteromorphic bivalent (h) and multivalent (m). × 1570.