more, protection is less. However, the radioresistance of lymphocytes appears to have increased due to the drug treatment, as a higher percentage survives at the early intervals. Hjort[8] also found a 3-fold increase in radioresistance of the lymphocytes with cysteamine; and Crouch and Overman[9] noticed protective effects of AET on peripheral blood in primates.

The protection of the lymphocytes in the present study may be attributed to the following reasons: 1. MPG protects the cells from direct killing, thus reducing the initial depletion; and 2. It protects the chromosomes by restitution, so that when the breaks rejoin, the normal structure is regained, which reduces abnormal divisions and mitotic deaths.

The second possibility serves to explain the failure to notice any significant cell depletion at 21 days in the drug-treated animals. Protection of the stem cell pool is also reflected in the fast recovery and restoration of normal cell count in the sublethally irradiated animals.

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Butanol extracts from myelin fragments. IV. Some interactions between 5-hydroxytryptamine and other neurotransmitters binding

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Summary. To examine the interaction between 5-HT and other neurotransmitters binding to the butanol extracts from myelin, double labelling experiments were done. The binding peaks of C^{14}·ACh and NA were clearly different from that of H^3 · 5-HT. At 5×10^{-7} M, binding of 5-HT, ACh, NA, GABA and DA was 62.7, 2.3, 7.0, 5.8 and 1.9 nmoles/mg protein, respectively. These results suggest that the 5-HT binding components of myelin butanol extracts may have high selectivity and specificity.

From the studies on the 5-HT binding to various membraneous structures of central nervous tissue, Marchbanks[1] and Fiszer and De Robertis[2] have reported the butanol extracts of myelin possessing a 5-HT binding capacity, but detailed biochemical observations were not performed. By Sephadex LH_{20} column chromatography[3] and SDS-urea gel electrophoresis[4], we demonstrated that the butanol extracts from myelin fragments showed binding affinity for C^{14}·5-HT, and these extracts contained basic protein, DM-20 of Agrawal[5] and proteolipid protein.

We also found that acetylcholine (ACh) and dopamine (DA) inhibited the binding of C^{14}·5-HT to these extracts, but noradrenaline (NA) and γ-aminobutyric acid (GABA) had no effect[6]. These results suggested that butanol extracts of myelin might have some interactions with other neurotransmitters as well as 5-HT. Based on these observations, we planned binding experiments of ACh, NA, GABA and DA to the butanol extracts from myelin fragments. In addition, the selectivity of 5-HT binding components is discussed.

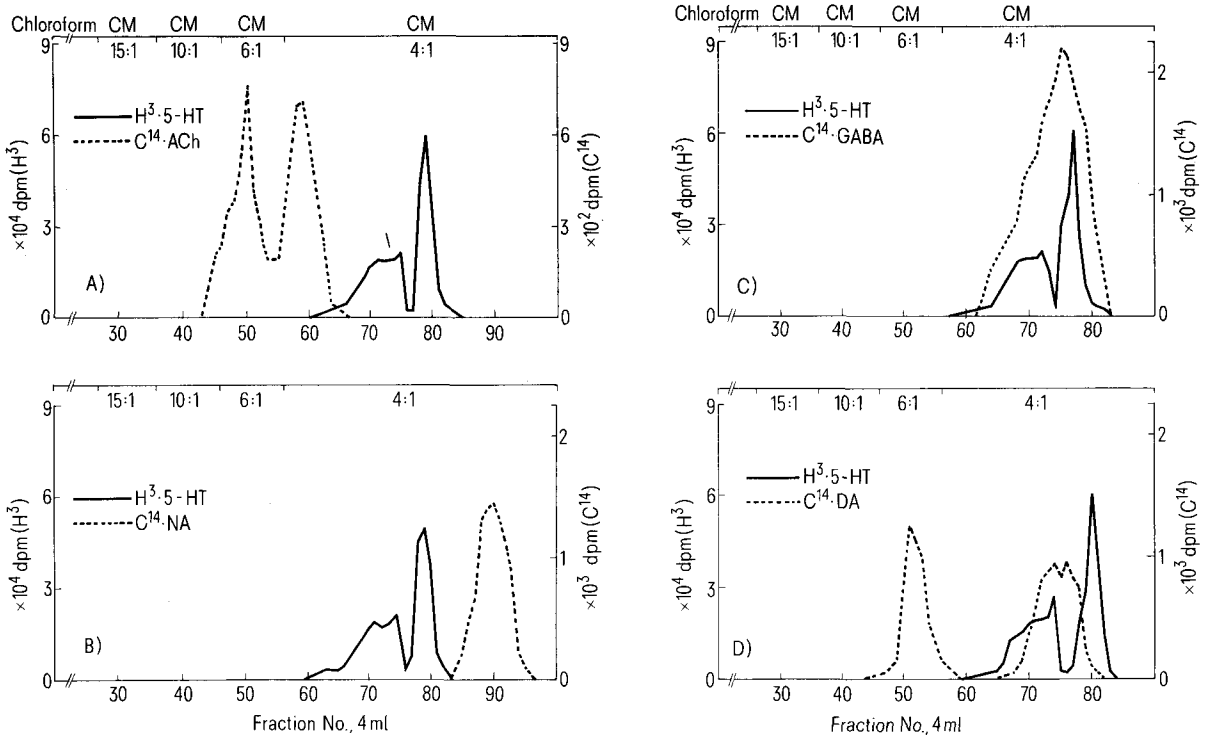
Materials and methods. Preparations of myelin fragments and butanol extracts were described previously[3,6]. Briefly, myelin fragments were isolated from the homogenate of rat brain stem (10% in 0.32 M sucrose) by the method of Whittaker et al.[7] and extracted with butanol-water mixtures. The resultant butanol phase was concentrated under N_2 to about one-third of its original volume (TE). An aliquot of TE (64 ml) was treated with water (14%, v/v) to dissolve the insoluble materials, and a 3 ml sample was simultaneously incubated at room temperature for 20 min with H^3·5-HT and 1 of the other neurotransmitters (C^{14}-labelled compound). After incubation, the mixtures were loaded on to a Sephadex LH_{20} column (2×30 cm). Stepwise elution was carried out with solvents of increasing polarity: 100 ml chloroform, 50 ml each of chloroform-methanol (CM) 15:1, 10:1 and 6:1, and then 200 ml of CM 4:1. Protein contents of the TE and collected fractions were assayed using the method of Lees and Paxman[8]. Radioactivity of the collected fractions was counted in a toluene/Triton X-100 emulsion phosphor. When the control experiment was done without protein moiety, the free counts of 5-HT and GABA appeared in the bound areas (11.7 and 21.5%, respectively) and in both cases they were subtracted from the experimental values. H^3·5-HT (27.6 Ci/mmole), C^{14}·NA (52.0 mCi/mmole), C^{14}·GABA (49.4 mCi/mmole) and C^{14}·DA (54.9 mCi/mmole) were obtained from New England Nuclear. C^{14}·ACh (10.2 mCi/mmole) was from Radiochemical Centre.

Results and discussion. In order to examine the interaction between 5-HT and other neurotransmitters binding, myelin butanol extracts were incubated simultaneously with H^3·5-HT and another C^{14}-labelled compound. The bound radioactivity of each of the compounds was determined by Sephadex LH_{20} column chromatography as described else-

Binding of several neurotransmitters to the butanol extracts from myelin fragments

Compound	nmoles/mg protein
5-Hydroxytryptamine	62.7±3.2
Acetylcholine*	2.3±0.3
Noradrenaline	7.0±1.3
γ-Aminobutyric acid	5.8±1.0
Dopamine*	1.9±0.4

Butanol extracts from myelin fragments were incubated for 20 min with 5×10^{-7} M of each of the neurotransmitters. After incubation, mixtures were chromatographed through a Sephadex LH_{20} column as described elsewhere and the bound radioactivity was measured (mean±SEM of 4 experiments). *In both cases, 2 binding peaks are combined.



Sephadex LH_{20} column chromatographic patterns of radioactivities of myelin butanol extracts. Extracts were simultaneously incubated with H^3·5-HT and another C^{14}-labelled neurotransmitter. Elution was carried out with the following solvents: 100 ml of chloroform, 50 ml each of chloroform-methanol (CM) 15:1, 10:1 and 6:1, and then 200 ml of CM 4:1. *A* H^3·5-HT; 5×10^{-7} M, C^{14}·ACh; 5×10^{-6} M. *B* H^3·5-HT; 5×10^{-7} M, C^{14}·NA; 1×10^{-6} M. *C* H^3·5-HT; 5×10^{-7} M, C^{14}·GABA; 1×10^{-6} M. *D* H^3·5-HT; 5×10^{-7} M, C^{14}·DA; 1×10^{-6} M.

where. C^{14}·ACh was eluted in 2 major fractions, i.e., the 1st corresponding to the area of CM 6:1 and the 2nd eluting in CM 4:1 solvent front. On the other hand, H^3·5-HT was eluted in the different fraction. The elution peak of C^{14}·NA also appeared in CM 4:1, but the double labelling experiments with C^{14}·NA and H^3·5-HT proved the clear difference of both binding components. The bound radioactivity of C^{14}·GABA was eluted with the solvent system of CM 4:1 and the binding peak was identically the same with that of H^3·5-HT. However, the inhibition studies[6] have revealed that 1000fold concentration of GABA has no effect on the binding of 5-HT to these extracts. The elution pattern of C^{14}·DA was similar in distribution to that seen with C^{14}·ACh but the 2nd peak was located in the same position of H^3·5-HT.

Godwin and Sneddon[9] reported the butanol extracts of rat brain stem-particulate fraction showing 5-HT binding capacity, and claimed the probability of those materials as receptor proteins. However, we pointed out that their particulate fraction contained considerable myelin fragments[3] and, moreover, the 5-HT binding capacity should originate from the butanol extracts of myelin[6]. They have also reported that 5-HT binding to those materials may involve a separate molecular species from that which apparently binds ACh, GABA or glycine.

Additionally, in the lipid extracts of nerve ending membranes of rat cerebral cortex, Fiszer De Plazas and De Robertis[10] indicated that a hydrophobic protein fraction binding C^{14}·GABA was eluted with the solvent of chloroform only.

To compare the binding capacity of myelin butanol extracts to 5-HT and other neurotransmitters, a relative binding value (i.e., amount of ligand bound/mg protein at 5×10^{-7} M) was used, since the displacement studies with 1000fold excess of unlabelled ligand indicated that only the 5-HT binding components were composed of specific and nonspecific portions but the remaining compounds were all nonspecific binding. The relative binding value of 5-HT, ACh, NA, GABA and DA was 62.7, 2.3, 7.0, 5.8 and 1.9 nmoles, respectively. These results show that the binding components present in the butanol extracts from myelin have higher affinity for 5-HT than other neurotransmitters.

In conclusion, all observations of the present studies suggest that the 5-HT binding components involved in the lipid extracts of myelin fragments may have high selectivity and specificity. In addition, from the studies on the recombination of myelin basic proteins and lipids[11], it is plausible that the nature of C^{14}·5-HT binding components might be basic proteins. Carnegie[12] has also proposed the specific interaction of myelin basic protein with 5-HT as the receptor and encephalitogenic protein.

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