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Structure-activity studies on a 5-hydroxytryptamine receptor of *Helix aspersa* neurones

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5-Hydroxytryptamine (5-HT) has a potent excitatory action on certain neurones of *Helix aspersa* (Kerkut & Walker, 1961) and there is good evidence for excitatory pathways mediated via the presynaptic release of 5-HT in snails (Kerkut, Ralph, Walker, Woodruff & Woods, 1970). This study investigates the structural requirements for the 5-HT receptor.

The electrical activity was recorded from identifiable neurones in the right parietal and visceral ganglia of the isolated snail brain, using the method described by Walker (1968). 5-HT excited all the cells used in the study, with a threshold response in the range of 2·5–25 pmol. All agonists tested were dissolved in snail Ringer and applied by addition to a 10 ml bath. Some compounds were initially dissolved in acid and then neutralized.

The potency of the compounds tested was expressed as the ratio of the molar doses of the compound and of 5-HT required to produce the same amount of depolarization of the membrane potential. The mean equipotent molar ratios, the range and the number of experiments are shown in Table 1. All compounds listed in this table caused

TABLE 1. *Excitatory activities of tryptamine analogues tested on specific neurones in the central nervous system of* Helix aspersa

Compound	Equipotent molar ratio range	Mean	Number of observations
5-Hydroxytryptamine	1	1	
α-Methyl-5-hydroxytryptamine	1–10	6·7	8
Bufotenine	13–130	45·6	14
5-Methoxytryptamine	42–210	120	10
Melatonin	172–8600	3088	8
Psilocin	2000–10,000	3500	5
Psilocybin	2000–10,000	4500	7
N,N-dimethyltryptamine	10,000–40,000	20,000	5

excitation. However, two tryptamine analogues tested showed an inhibitory effect. One compound, 5,6-dihydroxytryptamine, had a biphasic action, while the other, 6-hydroxytryptamine, always inhibited cell activity. The following compounds, in doses up to 1 μmol, failed to show any 5-HT-like activity: tryptamine, tyramine, N-methyltryptamine, 5-hydroxy-indole, 5-hydroxy-indolyl acetic acid, 5-methoxy-gramine, lysergic acid diethylamide, α-methyltryptamine, 5-methyltryptamine.

It is concluded that for potent 5-HT-like activity agonists should contain the following groups: an indole nucleus; either a hydroxyl or methoxy in the 5 position; a terminal unsubstituted nitrogen. Addition of methyl groups to the terminal nitrogen generally reduced potency.

REFERENCES

KERKUT, G. A., RALPH, K. L., WALKER, R. J., WOODRUFF, G. N. & WOODS, R. (1970). Excitation in the molluscan central nervous system, 105–117. In: *Excitatory Synaptic Mechanisms*, ed. Andersen, P. & Jansen, J. K. S. Oslo: Universitetsforlaget.

KERKUT, G. A. & WALKER, R. J. (1961). The effects of drugs on the neurones of the snail, *Helix aspersa. Comp. Biochem. Physiol.*, **3**, 143–160.

WALKER, R. J. (1968). Intracellular microelectrode recording from the brain of *Helix*, 342–345. In: *Experiments in Physiology and Biochemistry*, vol. **1**, ed. Kerkut, G. A. London: Academic Press.

The amiloride receptor

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The diuretic drug amiloride (3, 5-diamino-6-chloropyrazinoylguanidine) is a potent inhibitor of sodium transport in epithelia, and is believed to prevent the passive entry of sodium ions to the transport mechanism (Crabbé & De Weer, 1969; Gatzy, 1971) by combining with receptors in the mucosal surface of the epithelia. Alterations in the calcium concentration affect the potency of amiloride in a way which suggests that calcium may be involved in the interaction of amiloride with its receptor. Evidence for this was obtained as follows.

The sodium-dependent oxygen consumption of pieces of toad bladder epithelium was measured. Amiloride (10^{-4} M), a concentration more than sufficient to completely inhibit sodium transport, abolished the sodium-dependent oxygen consumption in solutions containing 1 mM calcium. In the absence of calcium, amiloride (10^{-4} M) had no effect on the sodium dependent oxygen consumption. This result clearly indicates the requirement for calcium, but does not locate its site of action.

Sodium transport in frog skin and toad bladder can be measured as the short circuit current (SCC). Unfortunately, when the epithelia are placed in calcium-free solutions with EGTA the cells become separated and SCC disappears. To overcome this difficulty the epithelia were bathed on the serosal surface with solutions containing calcium, while the mucosal solutions were calcium-free and contained EGTA. Under these conditions the epithelia continued to transport sodium. Dose-response curves relating SCC to amiloride concentration were obtained in the presence and absence of calcium. The curves obtained in the absence of calcium were very flattened compared to those obtained in the presence of calcium.

The data are consistent with the assumption that amiloride can block the entry of sodium to the transport mechanism only when it forms part of a ternary complex with