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Independent Regulation of Receptor-Mediated Cell Depolarization and Adenylate Cyclase by 5-Hydroxytryptamine (Serotonin) in Neuroblastoma Hybrid Cells

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5-Hydroxytryptamine (serotonin) accelerates or inhibits spontaneous neuronal firing in many areas of the brain (Bloom *et al.*, 1972). Responses are predominantly inhibitory and are mediated through both pre- and post-synaptic receptors, which have different regional distributions in brain and differ in their pharmacological properties (Haigler & Aghajanian, 1974; Aghajanian & Haigler, 1975). Stimulation of adenylate cyclase activity [ATP pyrophosphate-lyase (cyclizing), EC 4.6.1.1] by 5-hydroxytryptamine has been demonstrated in mammalian brain (Enjalbert *et al.*, 1978*a*,*b*) and is mediated by post-synaptic 5-hydroxytryptamine receptors (Enjalbert *et al.*, 1978*a*). The possibility of multiple 5-hydroxytryptamine-receptor functions being expressed in cultured neuroblastoma hybrid cells was investigated.

Cholinergic hybrid cell lines were screened for 5-hydroxytryptamine-dependent regulation of adenylate cyclase and acetylcholine release. Results are presented for the NG108-15 neuroblastoma × glioma hybrid (Klee & Nirenberg, 1974) and the NCB-20 neuroblastoma × brain of foetal Chinese hamster hybrid (Minna *et al.*, 1975). Electrophysiological data were obtained by using methods described previously (Christian *et al.*, 1978). Iontophoretic application of 5-hydroxytryptamine produced depolarizing responses in NG108-15 and NCB-20 hybrid cells with the generation of action potentials, which were neither inhibited nor mimicked by LSD. Results in NG108-15 cells confirmed the findings of Christian *et al.* (1978). Repeated application of 5-hydroxytryptamine at a frequency of 0.5 Hz desensitized the receptors with a decrease in the depolarizing response and eventual loss of action potentials. Both hybrid cell lines are synaptically competent and formed stable synapses with myotubes in primary cultures from Fisher-rat hind limb. Application of 5-hydroxytryptamine to the hybrid cells in co-culture produced an increase in acetylcholine release, which was shown by the increase in the frequency of miniature end-plate potentials and evoked muscle action potentials. These effects were not inhibited by a saturating concentration of LSD in either cell line.

Adenylate cyclase activity was determined by a modification of the method of Salomon *et al.* (1974). A 5-hydroxytryptamine-dependent increase in adenylate cyclase activity of 75–115% was observed in whole-cell homogenates of the NCB-20 hybrid cells. The concentration of 5-hydroxytryptamine for half-maximum activation ($K_{act.}$) was 0.4–0.8 μM and was unaffected by the addition of 50 μM-ascorbate or 100 μM-pargyline to the reaction mixture. LSD produced a partial inhibition of the 5-hydroxytryptamine-dependent stimulation of adenylate cyclase (K_i = 10nM), and also produced a small

Abbreviation used: LSD, D-lysergic acid diethylamide.

Table 1. *Properties of the receptor-mediated functions of 5-hydroxytryptamine in two neuroblastoma hybrid cell lines*

The NG108-15 cell line is a hybrid of mouse neuroblastoma and rat glioma cells. The NCB-20 cell line is a hybrid of mouse neuroblastoma and brain of 18-day-foetal Chinese hamster. Depolarizing responses were detected by intracellular recording techniques during iontophoretic application of serotonin. Adenylate cyclase activity was determined in whole cell homogenates.

Experiment	NG108–15	NCB-20
Depolarizing responses		
5-Hydroxytryptamine	++	++
LSD	None	None
Stimulation of adenylate cyclase		
5-Hydroxytryptamine	None	++
LSD	None	+
Inhibition of response to 5-hydroxytryptamine by LSD		
Depolarizing responses	None	None
Adenylate cyclase stimulation		+
Desensitization of 5-hydroxytryptamine-dependent response		
Depolarizing response	++	++
Adenylate cyclase stimulation		None

ncrease (41%) in basal enzyme activity ($K_{act.} = 12$ nM). This effect is characteristic of a partial agonist and is similar to the response of postsynaptic 5-hydroxytryptamine receptors coupled to adenylate cyclase in mammalian brain (Enjalbert *et al.*, 1978*b*). The production of cyclic AMP in whole-cell homogenates of NCB-20 hybrid cells was shown to increase linearly with time for 16 min in the presence or absence of 10 μM-5-hydroxytryptamine or 100 nM-LSD, indicating no rapid desensitization of 5-hydroxytryptamine receptors. No 5-hydroxytryptamine-dependent activation of adenylate cyclase was observed in NG108-15-cell homogenates.

Table 1 summarizes the differences in the observed responses to 5-hydroxytryptamine in the two cell lines, showing that regulation of adenylate cyclase activity and acetylcholine release are mediated by different species of 5-hydroxytryptamine receptors. The two receptor populations are distinguished by their functional response, their sensitivity to LSD and desensitization. The data suggest that activation of adenylate cyclase does not affect acetylcholine release, and conversely that 5-hydroxytryptamine-dependent cell depolarization does not regulate adenylate cyclase activity in the hybrid cells tested.

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