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Stimulation of adenylate cyclase activity in monkey anterior limbic cortex by serotonin

HO SAM AHN and MAYNARD H. MAKMAN

Department of Biochemistry and Molecular Pharmacology, Albert Einstein College of Medicine, Bronx, N.Y. 10461 (U.S.A.)

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Serotonin (5-hydroxytryptamine, 5-HT) has been implicated in a wide variety of brain functions[8] and while its precise role is poorly understood, there is persuasive evidence that it functions as a neurotransmitter in the central nervous system (CNS)[1]. The findings that 5-HT was capable of increasing the accumulation of cyclic AMP in brain slices of various animal species or stimulating adenylate cylcase activity in cell-free preparations from most brain regions of immature rats[6,17] suggest the possibility that the various effects of 5-HT may be mediated at least in part by cyclic AMP. Recent electrophysiological and pharmacological studies[1,17] suggested the occurrence of more than one type of 5-HT receptors in the mammalian CNS. Further, regional differences of adenylate cyclase in response to 5-HT were observed in studies employing brain slices or cell-free preparations[15,16]. In previous studies both anterior limbic cortex (ALC) and frontal cortex (FC) of Cebus monkey were found to contain dopamine-stimulated adenylate cyclase activity[3,12,13]. However, D-lysergic acid diethylamide (LSD) stimulated activity in ALC but not in FC[2]. Since LSD has been reported to be a mixed 5-HT as well as a dopamine agonist–antagonist, it seemed possible that 5-HT-sensitive adenylate cyclase activity might be present in ALC. Also, there might be present different types of 5-HT receptors associated with ALC and FC of primate brain since both of these two brain regions are known to receive serotonergic innervation[1] and to possess a very high density of serotonin binding sites[1,4]. We report here the ability of 5-HT and 5-HT agonists to stimulate adenylate cyclase activity in ALC but not FC of Cebus monkey and the differentiation of 5-HT from dopamine effects in ALC with use of antagonists.

Ten monkeys *(Cebus appella)* ranging in age between 2 and 5 years were used. The animals were anesthetized with Nembutal (50–70 mg/kg i.p.) prior to sacrifice and ALC (anterior portion of areas 24 and 25 of Walker and Brodmann; see ref. 18) and FC (frontal orbital regions) removed as previously described[3,13]. Adenylate cyclase assay and cyclic AMP determination were performed as previously described[3] except that ALC and FC were homogenized respectively in 60 and 40 parts of the homogenization buffer (w/v). Each experiment involved triplicate adenylate cyclase incubations for every condition studied, with single or replicate determinations of cyclic AMP.

TABLE I

Comparison of effects of serotonin and related agents on adenylate cyclase activities in anterior limbic cortex and frontal cortex of Cebus monkey

Agents added	*Conc. (μM)*	*Anterior limbic cortex*		*Frontal cortex*	
		pmoles cAMP formed per mg protein/5 min	*% change*	*pmoles cAMP formed per mg protein/5 min*	*% change*
None		113 ± 7 (3)		174 ± 8 (5)	
Serotonin	0.1	141 ± 9 (1)[a]	25	N.A.	
Serotonin	1	167 ± 5 (3)[c]	48	186 ± 13 (4)	7
Serotonin	10	191 ± 11 (3)[c]	69	158 ± 12 (5)	—9
Serotonin	100	226 ± 18 (3)[c]	100	143 ± 10 (5)[a]	—18
Serotonin	500	215 ± 10 (1)[b]	90	103 ± 10 (1)[c]	—41
LSD	1	192 ± 14 (2)[c]	70	150 ± 8 (5)[a]	—14
LSD	10	209 ± 9 (3)[c]	85	165 ± 12 (5)	—5
Capsaicin	10	205 ± 7 (3)[c]	81	179 ± 7 (1)	3
Psilocin	10	150 ± 7 (2)[b]	33	N.A.	
Quipazine	50	124 ± 6 (3)	10	172 ± 6 (1)	—1
Methysergide	10	124 ± 6 (2)	10	164 ± 9 (1)	—6
Cyproheptaline	10	119 ± 5 (2)	5	N.A.	

Each value represents the mean ± S.E.M. as described in the text (number of separate experiments in parentheses). Values were significantly different from the basal activities at $P < 0.05$ for [a], at $P < 0.005$ for [b], and at $P < 0.001$ for [c]. N.A. not assayed.

A Student's *t*-test was used to determine the significance of difference in mean values between basal and agent-stimulated activities.

Serotonin stimulated in a dose-dependent manner adenylate cylcase activity in homogenates of ALC of Cebus monkey (Table I). A significant stimulation (25%) of ALC cyclase was observed for a concentration of 5-HT as low as 0.1 m*M* and half-maximal activation was produced by approximately 1 μM 5-HT with a maximal stimulation at about 100 μM (Table I). In addition enzyme activity in ALC was also significantly stimulated by LSD, psilocin and capsaicin (8-methyl-*N*-vanillyl-6-nonenamide), a compound reported to interact with central warm sensitive 5-HT receptors[11]. Our findings are in agreement with previous reports[11,17] of activation by these indoles of adenylate cyclase in cell-free preparations of superior colliculi and several other regions of rat brain. On the other hand, quipazine (2-(1-piperazinyl) quinoline maleate), also known to stimulate central 5-HT receptors[14] but without effect on adenylate cyclase of newborn rat colliculi[9,10], was without effect in monkey ALC (Table I).

In marked contrast to ALC, adenylate cyclase activity of FC was not stimulated by 5-HT, LSD or capsaicin (Table I). Rather, at certain concentrations, 5-HT (100 μM) and LSD (1 μM) significantly inhibited the basal activity of FC enzyme. The nature of this inhibition at present is not known. These data, together with the results mentioned above, indicate regional differences in response to 5-HT and its agonists.

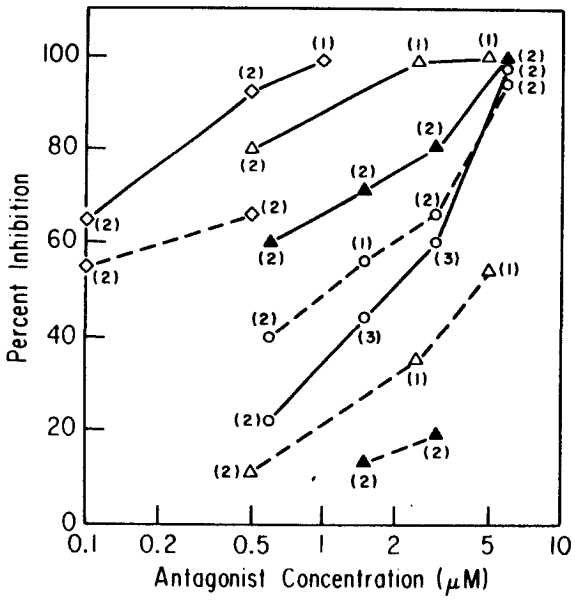

Fig. 1. Influence of varying concentrations of blocking agents on serotonin-, dopamine- or LSD-stimulated activity of Cebus monkey anterior limbic cortex. Each point represents the per cent inhibition (the mean of 3–9 incubations with standard errors less than 10% inhibition) by antagonist of the response to serotonin, dopamine or LSD. The basal adenylate cyclase activity was 149 ± 4 pmoles cyclic AMP formed/mg protein/5 min (the mean ± S.E.M. for 10 separate experiments.) Stimulated activities were 246 ± 14 (5 expts) in the presence of 30 μM serotonin (○); 395 ± 20 (5 expts) in the presence of 30 μM dopamine (▲); 260 ± 12 (3 expts) in the presence of 5 μM dopamine (△); and 228 ± 4 pmoles cyclic AMP formed/mg protein/5 min (3 expts) in the presence of 1 μM LSD (◇). Data are presented for the antagonists, methysergide (-----) and haloperidol (——).

While these results suggest the presence of 5-HT-sensitive adenylate cyclase in ALC, it could be possible that 5-HT agonists cross-interact with dopamine receptors, with resultant activation of cyclase activity, since the ALC has a very sensitive dopamine receptor-adenylate cyclase[12,13]. In order to further elucidate the type of receptor mediating 5-HT stimulation of adenylate cyclase in ALC, the effects of 5-HT- and dopamine-blocking agents were examined. Although at concentrations equal to or greater than 1/10 that of 5-HT, methysergide, a 5-HT antagonist, and haloperidol, a dopamine receptor blocking agent, were approximately equally effective in antagonizing the 5-HT-stimulated activity, with a complete inhibitions of 5-HT stimulation at concentrations 1/5 that of 5-HT; at lower concentrations, methysergide was more effective than haloperidol (Fig. 1). Haloperidol was much more effective than methysergide in blocking the dopamine-stimulated activity at all concentrations used (Fig. 1). These data indicate the presence of a separate 5-HT-sensitive adenylate cyclase in addition to the dopamine sensitive enzyme in the ALC. On the other hand, the LSD-stimulated activity was nearly equally inhibited by methysergide or haloperidol at concentrations which resulted in selective antagonistic effects on 5-HT- and dopamine-stimulated activities (Fig. 1). These results are consistent with the stimulation of both 5-HT and dopamine receptors by LSD in this region.

The main finding of the present study is the demonstration of 5-HT-stimulated adenylate cyclase in the ALC but not in the FC of Cebus monkey. The maximal stimulation (about 100%) of cyclase activity by 5-HT in the ALC of young adult monkeys was greater than that reported for colliculi (reportedly the most responsive region) and several other brain areas of adult rats[16]. In the monkey system LSD, psilocin and capsaicin were also stimulatory. The findings for the monkey ALC system differed somewhat from that of adult rat colliculi[9,16] in that the ALC was not stimulated by cyproheptadine and was relatively more responsive to LSD. Although at higher concentrations the antagonists were not specific in agreement with their known significant affinity for both dopamine and 5-HT binding sites[4,5], at lower concentrations methysergide was more effective than haloperidol in blocking the 5-HT stimulated activity whereas the reverse was true for inhibition of the dopamine-stimulated activity, thus indicating two separate adenylate cyclases responsive to dopamine and 5-HT, respectively. Since both FC and limbic cortex are reported to have serotonergic innervation and specific 5-HT binding sites[1,7], the differential response to 5-HT in FC and ALC suggests that 5-HT receptors in ALC but not in FC may be coupled to adenylate cyclase. Alternatively, the coupling of 5-HT receptors to adenylate cyclase in FC may be more labile to homogenization. The lack of effect of quipazine on enzyme activity in ALC might be accounted for if quipazine were to interact with 5-HT receptors not coupled to adenylate cyclase. Different responses to classical 5-HT receptor antagonists of 5-HT receptors of raphe nucleus and brain stem were previously reported in electrophysiological studies[1]. The 5-HT receptor associated with adenylate cyclase found in the ALC differs from raphe or amygdala 5-HT receptors[1] which mediate the inhibition of neuronal firing rate by iontophoretically applied 5-HT and LSD in that it is readily inhibited by methysergide, but resembles more closely 'post-synaptic' 5-HT receptors as characterized by [^{3}H]5-HT binding studies[4]. The present studies together with previous findings[2] indicate that both dopamine- and 5-HT-sensitive adenylate cyclase systems may serve as biochemical substrates for LSD. Further studies will be required to ascertain the relative significances of these two systems in the action of LSD. Because of the relatively large response of ALC cyclase to 5-HT and related compounds, together with larger amounts of tissue available compared with rat colliculi, the monkey ALC provides a suitable in vitro model for detailed pharmacologic studies in 5-HT receptors.

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1 Aghajanian, G. K., Haigler, H. J. and Bennett, J. L., Amine receptors in CNS. III 5-Hydroxytryptamine in brain. In L. L. Iversen, S. D. Iversen and S. Snyder (Eds.), *Handbook of Psychopharmacology, Vol. 6*, Plenum Press, New York, 1975, pp. 63–95.

2 Ahn, H. S. and Makman, M. H., Interaction of LSD and other hallucinogens with dopamine-sensitive adenylate cyclase in primate brain: regional differences, *Brain Research*, (1978) in press.

3 Ahn, H. S., Mishra, R. K., Demirjian, C. and Makman, M. H., Catecholamine sensitive adenylate cyclase in frontal cortex of primate brain, *Brain Research*, 116 (1976) 437–454.

4 Bennett, J. P., Jr. and Snyder, S. H., Serotonin and lysergic acid diethylamide binding in rat brain membranes: relationship to postsynaptic serotonin receptors, *Molec. Pharmacol.*, 12 (1976), 373–389.
5 Creese, I., Burt, D. R. and Snyder, S. H., The dopamine receptor: differential binding of D-LSD and related agents to agonist and antagonist states, *Life Sci.*, 17 (1976) 1715–1720.
6 Daly, J., *Cyclic Nucleotides in the Nervous System*, Plenum Press, New York, 1977.
7 Enna, S. J., Bennett, J. P., Jr., Bylund, D. B., Creese, I., Burt, D. R., Charness, M. E., Yamamura, H. I., Simantov, R. and Snyder, S. H., Neurotransmitter receptor binding: regional distribution in human brain, *J. Neurochem.*, 28 (1977) 233–236.
8 Green, A. R. and Grahame-Smith, D. G., 5-Hydroxytryptamine and other indoles in the central nervous system. In L. L. Iversen, S. D. Iversen and S. H. Snyder (Eds.), *Handbook of Psychopharmacology, Vol. 3*, Plenum Press, New York, 1975, pp. 169–245.
9 Hamon, M., Bourgoin, S. and Enjalbert, A., Biochemical regulatory mechanisms of serotonergic neurons in the rat brain. In P. O. Hubinont, M. L.Hermite and C. Robyn (Eds.), *Progress in Reproductive Biology, Vol. 2, Clinical Reproductive Neuroendocrinology*, Karger, New York, 1977, pp. 25–40.
10 Hamon, M., Bourgoin, S., Enjalbert, A., Bockaert, J., Hery, F., Ternaux, J. P. and Glowinski, J., The effects of quipazine on 5-HT metabolism in the rat brain, *Arch. Pharmacol.*, 294 (1976) 99–108.
11 Jancso, G. and Wollemann, M., The effect of capsaicin on the adenylate cyclase activity of rat brain, *Brain Research*, 123 (1977) 323–329.
12 Makman, M. H., Actions of cyclic AMP and relationship to transmitter function in nervous tissue. In G. Litwack (Ed.), *Biochemical Actions of Hormones, Vol. 4*, Academic Press, New York, 1977, pp. 407–496.
13 Mishra, R. K., Demirjian, C., Katzman, R. and Makman, M. H., A dopamine sensitive adelylate cyclase in anterior limbic cortex and mesolimbic regions of primate brain, *Brain Research*, 96 (1975) 395–399.
14 Rodriguez, R., Rojas-Ramirez, J. A. and Drucker-Colin, R. R., Serotonin-like actions of quipazine on the central nervous system, *Europ. J. Pharmacol.*, 24 (1973) 164–171.
15 Skolnick, P., Huang, M., Daly, J. and Hoffer, B., Accumulation of adenosine 3′,5′-monophosphate in incubated slices from discrete regions of squirrel monkey cerebral cortex: effect of norepinephrine, serotonin and adenosine, *J. Neurochem.*, 21 (1973) 237–240.
16 Von Hungen, K. and Roberts, S., Neurotransmitter-sensitive adenylate cyclase systems in the brain. In S. Ehrenpreis and I. J. Kopin (Eds.), *Reviews of Neuroscience, Vol. 1*, Raven Press, New York, 1974, pp. 231–281.
17 Von Hungen, K., Roberts, S. and Hill, D. F., Serotonin-sensitive adenylate cyclase in immature rat brain, *Brain Research*, 84 (1975) 257–267.
18 Walker, A. E., A cytoarchitectural study of the prefrontal area of the macaque monkey, *J. comp. Neurol.*, 73 (1940) 59–86.