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COMMUNICATIONS

Localization of dopamine receptors in the rat cerebral cortex

M. TRABUCCHI, S. GOVONI, G. C. TONON, P. F. SPANO, *Department of Pharmacology and Pharmacognosy, University of Milan, 20129 Milan, Italy*

Only four major dopaminergic neuronal tracts had been described until recently in the mammalian brain: the nigrostriatal tract, the retinal tract, the tuberoinfundibular tract and the mesolimbic tract (Ungerstedt, 1971). Through consideration of the known anatomy and physiology of these tracts, several investigators have concluded that the mesolimbic dopaminergic tract may be the most likely candidate for a relevant role in the schizophrenic process (Matthysse, 1973; Stevens, 1973; Torrey & Peterson, 1974). However, the presence of dopaminergic terminals in some regions of the rat cerebral cortex has been documented more recently (Thierry, Blanc & others, 1973; Thierry & Glowinski, 1973; Lindvall & Bjorklund, 1974; Hökfelt, Ljungdahl & others, 1974b; Hökfelt, Fuxe & others, 1974a; Berger, Tassin & others, 1974). Additional observations indicate that the dopamine terminals of the cerebral cortex originate from the same areas (A9 and A10) where the nigrostriatal and mesolimbic terminals have their origin (Lindvall, Bjorklund & others, 1974). Moreover, since the cerebral cortex is most likely associated with some of the major symptoms of schizophrenia, such as disturbances of thinking and symbolic processes (Kety & Matthysse, 1972), the discovery of cortical dopamine terminals gives further support to the hypothesis of a role of dopamine in the pathogenesis of schizophrenia.

On the other hand, adenylate cyclases, which are specifically sensitive to dopamine, have also been described in homogenates of bovine superior cervical ganglia (Kebabian & Greengard, 1971), calf and rat retinas (Brown & Makman, 1972), rat caudate nucleus (Kebabian, Petzold & Greengard, 1972), and n. accumbens and tuberculum olfactorium in the mesolimbic system (Horn, Cuello & Miller, 1974). These findings have led to the suggestion that in these areas the dopamine-sensitive adenylate cyclase and the dopamine receptor may be related, and that the physiological effects of dopamine could be mediated by cyclic adenosine monophosphate (cyclic AMP). Hunger & Roberts (1973) have recently reported the presence of a dopamine stimulated adenylate cyclase in a cell-free preparation of rat cerebral cortex. However, no specific dopamine-sensitive adenylate cyclase activity has been described so far in homogenates of discrete areas of the cerebral cortex. We have, therefore, examined areas of the cerebral cortex to establish the existence of a specific dopamine sensitive adenylate cyclase activity.

Male Charles River rats, 110–130 g, were used. Adenylate cyclase activity was measured in homogenates of the striatum and two cortical regions, frontal neocortex and entorhinal cortex, according to Kebabian & others (1972) with some modifications. After 3 min of incubation the reaction was stopped by boiling the samples for 3 min. The cyclic AMP was purified and assayed using the method for cAMP dependent protein kinase described by Kuo & Greengard (1972). Adenylate cyclase activity was measured by adding several concentrations of dopamine and noradrenaline. Section of the various cortical areas was according to Berger & others (1974). Protein was measured according to Lowry, Rosebrough & others (1951).

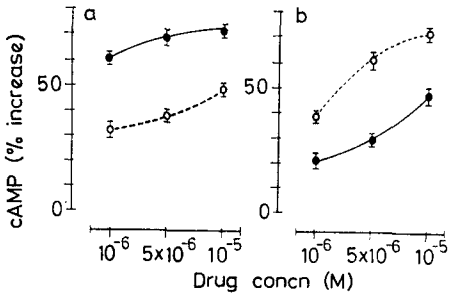

FIG. 1. Differential effects of noradrenaline (●) and dopamine (○) on cyclic AMP formation in (a)-neocortex and (b)-entorhinal cortex. Values expressed as mean ± s.e. of at least 6 determinations. The basal values are 68 ± 3·2 and 60 ± 5·8 pmol cAMP formed mg^{-1} protein min^{-1} respectively in entorhinal cortex and neocortex.

In the entorhinal cortex (Fig. 1) the percentage increase of adenylate cyclase activity was much larger after addition of dopamine than after equimolar concentrations of noradrenaline. But in the neocortex the reverse occurred. Only at higher concentrations (10^{-5} M) did the two curves tend to approach. For a better understanding of the function of dopamine-dependent adenylate cyclase of the cortex, the effect of the specific dopamine antagonist haloperidol was also examined. Haloperidol at a low concentration (10^{-6}M) completely antagonized the stimulation of cyclic AMP formation elicited by dopamine in the entorhinal cortex (Table 1), as has been found with striatal homogenates (Clement-Cormier, Kebabian & others, 1974). On the other hand, after *in vivo* injection of reserpine

Table 1. *Adenylate cyclase activity in brain entorhinal cortex and striatum after various* in vitro *and* in vivo *treatments.* The values are expressed as pmol cAMP formed mg^{-1} protein min^{-1}.

Drug	Entorhinal cortex	Striatum
Controls	65 ± 1·8	279 ± 10
Dopamine (5 μM)	98 ± 5·2*	463 ± 16*
Haloperidol (1 μM)	60 ± 2·5	247 ± 21
Haloperidol (1 μM) + dopamine (5 μM)	70 ± 4·1	290 ± 5·7
Reserpine	58 ± 5·5	265 ± 12
Reserpine + dopamine (5 μM)	118 ± 6·3*	624 ± 24*

* $P < 0{\cdot}001$ in respect to controls and $< 0{\cdot}01$ in respect to dopamine values. Values of cAMP formed are means (± s.e.m.) of at least 12 determinations. Reserpine was injected 20 h before killing (5 mg kg^{-1}, i.p.).

(5 mg kg^{-1}, i.p., 20 h before) a significant increase of dopamine-stimulated formation of cyclic AMP was detected in the entorhinal cortex compared to saline-injected rats. These data indicate that the dopamine receptor in the cortex becomes supersensitive after chemical denervation as has been found in striatum, the limbic system and retina (Kumakura, Carenzi & others, 1975; Trabucchi & Spano, 1975).

In conclusion, our data suggest the possibility that dopamine-dependent adenylate cyclase is related to the dopamine receptor in some cortical areas of the rat, and that the physiological effects of dopamine could be mediated in this area by cyclic AMP.

Moreover, the evaluation of dopamine-dependent adenylate cyclase in the cortex furthers the understanding of the mode of action of antipsychotic drugs and the dopaminergic mechanisms underlying the pathogenesis of schizophrenia. The block by haloperidol of the stimulation of cyclic AMP elicited by dopamine in the cortex and the supramaximal response to dopamine after *in vivo* treatment with reserpine—which indicates that dopamine receptor supersensitivity may be operant in this area of the brain—substantiates these views. The block of striatal dopamine receptors by neuroleptics, which gives rise to the extrapyramidal side effects of these drugs through a disinhibition of striatal cholinergic neurons (Trabucchi, Cheney & others, 1974), may now be reconsidered in a new light. Recently, it has been demonstrated that haloperidol increases the rate of dopamine synthesis in the cortex as it does in the striatum (Scatton, Thierry & others, 1975). On the other hand, we have recently shown that LSD is capable of stimulating the formation of cyclic AMP in the limbic cortex as much as in other areas of the brain where dopamine terminals have been described (Spano, Kumakura & others, 1975).

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