Cerebral phosphodiesterase and the dopamine receptor

During a study of the kinetics and inhibition of cerebral phosphodiesterase (adenosine-3',5'cyclic phosphate, adenosine-5'phosphate, 3'phosphohydrolase, E.C.3.1.4.C), it became apparent that apomorphine and bulbocapnine, agents that are believed to interact with cerebral dopamine receptors (Ernst & Smelik, 1966; Ernst, 1969), were more potent inhibitors of phosphodiesterase than the widely used methylxanthines. In this communication we report our findings with these and related compounds.

Phosphodiesterase activity was estimated with labelled cyclic AMP (adenosine-8 labelled [^{3}H] cyclic phosphate) as substrate. Rate of hydrolysis of [^{3}H]cyclic AMP to [^{3}H] 5'-AMP was determined after removal of the labelled product by barium sulphate precipitation (Pöch, 1971). Incubations were carried out in the presence of 5×10^{-3} AMP to minimize the conversion to adenosine by the 5'nucleotidase activity of the enzyme preparation. The enzyme used in all experiments was prepared from rat cerebral cortex by homogenization with 50 mM tris-5 mM $MgCl_2$. Following centrifugation at 5000 *g* the relatively particle-free supernatant was suitably diluted and used directly in the assay. Under the conditions used the reaction rate was linear for at least 30 min. In agreement with earlier reports (Brooker, Thomas & Appleman, 1968; Beavo, Hardman & Sutherland, 1970) kinetic analyses of the cerebral enzyme revealed a form with an apparent Km of approximately $2{\cdot}0 \times 10^{-6}$M in addition to a form with a Km of about 1×10^{-4}M. In all experiments low (1–3 μM cyclic AMP) substrate levels that are within the physiological range were used, the data presented representing inhibition of the low Km enzyme. Results are expressed in terms of I_{50}, the concentration of drug required to inhibit the enzyme by 50%.

The results presented in Fig. 1 show that papaverine, apomorphine and bulbocapnine are much more potent than the methylxanthines theophylline and caffeine which are classically associated with phosphodiesterase inhibition. Papaverine has previously been shown to be a potent inhibitor of phosphodiesterase in brain (Goldberg, Lust & others, 1970) and in other tissues (Kukovetz & Pöch, 1970).

In view of these findings and since bulbocapnine and apomorphine (Ernst & Smelik, 1966; Ernst, 1969) and possibly papaverine (Loizzo, Scotti & Longo, 1971) are believed to interact with cerebral dopamine receptors, we have also examined the effect of dopamine and the other appropriate β-phenethylamine moieties of the above

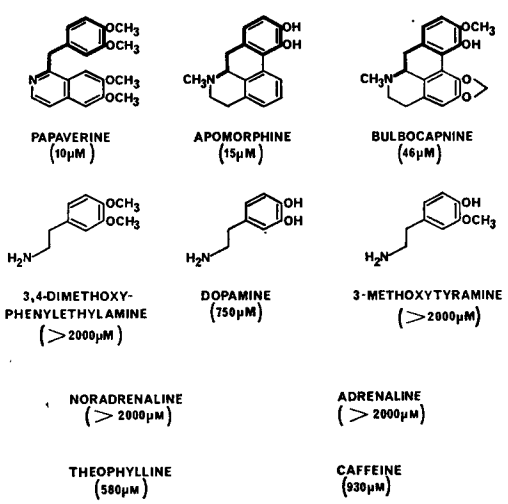

FIG. 1. Inhibition of rat cerebral cortical phosphodiesterase. Values in parentheses indicate the concentration of drug required to inhibit the enzyme by 50%.

compounds (3-methoxytyramine and dimethoxyphenylethylamine) on cerebral phosphodiesterase activity. It is clear that, although less potent than papaverine, bulbocapnine or apomorphine, dopamine is of the same order of potency as caffeine and it is significant that introduction of a β-OH group to the catecholamine results in the inactive noradrenaline (Fig. 1).

It has been suggested that tetrahydropapaveroline may be an important metabolite of dopamine, particularly when tissue dopamine levels are high e.g. following L-dopa administration (Walsh, Davies & Yamanaka, 1970). It is likely that such a metabolite and indeed tetrahydroxynoraporphine, a further putative metabolite (Sourkes, 1971), because of their similarity to papaverine and apomorphine are potent phosphodiesterase inhibitors.

Recent work by Kebabian, Petzold & Greengard (1972) has indicated that dopaminergic transmission in the caudate nucleus may be mediated by cyclic AMP since both dopamine and apomorphine stimulate the adenyl cyclase activity of this brain region. Moreover, Florendo, Barrnett & Greengard (1971) have shown that most of the phosphodiesterase in slices of rat parietal cortex is located in the region of the synaptic membrane and, in cases where pre- and post-synaptic elements of synapses could be identified, the enzyme activity appeared to be exclusively located post-synaptically.

It appears tenable, therefore, that the dopamine receptor may be closely associated with a form of phosphodiesterase, and agents interacting with such a receptor may alter cyclic AMP metabolism and as a result possibly modify post-synaptic electrical events (Greengard, McAfee & Kebabian, 1972; Siggins, Hoffer & Bloom, 1971).

It is clear, however, that cerebral phosphodiesterase is not a simple molecular entity (Uzunov & Weiss, 1972) but probably exists in several molecular forms each of which may be differently distributed in various brain regions and susceptible to inhibitors to varying degrees. Separation of forms of phosphodiesterase in the caudate nucleus and careful examination of the effects of the agents described above would appear to be a fruitful line of future research.

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