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APOMORPHINE-INDUCED ACCUMULATION OF CYCLIC AMP IN ISOLATED RETINAS OF THE RABBIT

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It has been shown recently that homogenates of calf and rat retinas[1] and homogenates of rabbit retinas[2] contain an adenylate cyclase sensitive to dopamine and apomorphine[2]. A close relationship between adenylate cyclase and dopamine receptor might therefore be suggested as in homogenates of rat caudate nucleus[3]. Although the adenylate cyclase activity in homogenates of retinas seems to be very sensitive to low doses of dopamine (and apomorphine[2]), the use of intact cell preparations, rather than broken cell preparations, was found to be very appropriate to study the mechanism of action, at cellular level, of apomorphine, mescaline and amantadine, potential stimulators of dopamine receptors in vivo[4,5,6].

Material and methods.

Adult rabbits were shot in the neck and the eyes were enucleated immediately. The anterior section of the eye was removed and the retina isolated by gently detaching it from the choroid with a small brush. The optic nerve was cut and the loose retina immersed in a Krebs-Ringer bicarbonate medium (pH 7.4) at 4°C. Tissue was then preincubated for 40 min at 35°C in 20 ml of the Krebs-Ringer bicarbonate buffer, which was gassed continuously with 95 % O_2-5 % CO_2. At the end of the 40 min preincubation, each retina was transferred to 1.0 ml fresh medium containing 5 mM theophylline, and 0.1 ml control medium or medium with various pharmacological agents was added. Incubations were terminated after 10 min at 35°C by adding 0.1 ml 50 % TCA followed by homogenization. The homogenate was centrifuged, dissolved in 2.0 ml of NaOH 0.5 N containing 5 % SDS[7] and used for protein determination[8]. 1.0 ml of the supernatant was neutralized with 0.1 ml TRIS base 3.5 M and Dowex 50 W-X-8 columns[9] used for purifi-

cation and isolation of cyclic AMP. 2 aliquots (0.25 ml) of the cyclic AMP fraction (3 ml) from the columns were evaporated to dryness for the saturation assay method[10].

Results and discussion

As shown in table 1, apomorphine was found to be a better stimulator than dopamine of the adenylate cyclase activity in intacts retinas of the rabbit, in contrast to experiments performed with tissue homogenates[2]. The effects of apomorphine and dopamine were blocked by haloperidol (Table 1), indicating that both agents were stimulating the same hypothetical dopamine receptor. The large accumulation of cyclic AMP in response to apomorphine in intact retinas of the rabbit was also in contrast with the small effect of the drug observed in intact calf retina in the same experimental conditions[11].

As apomorphine was found to be more potent in intact cells than in homogenates, the effects of mescaline and amantadine, both inactive in tissue homogenates[12], were also investigated. As shown in table 2, both agents were ineffective. Thus the mechanism of action of these drugs, at cellular or subcellular level, has not yet been clarified. It is interesting to consider, why dopamine is more active in homogenates, while apomorphine seems to be more active in intact cells. It would appear possible that apomorphine (and other

Table 1. Effect of haloperidol on the dopamine- and apomorphine- induced accumulation of cyclic AMP in intact retinas of the rabbit.

		Δ pmoles cAMP . mg protein^{-1} *	
Experiment		- haloperidol	+ 500 µM haloperidol
I	100 µM dopamine	26.26 ± 4.59	10.03 ± 6.73
II	100 µM apomorphine	43.11 ± 10.18	2.08 ± 3.41

* Cyclic AMP is expressed as the increase (Δ ± S.E.M.) in cAMP above control values determined each time for experiment I and for experiment II with the same number of retinas (5).

Table 2. Cyclic AMP concentration in intact retinas of rabbit in response to dopamine, apomorphine, mescaline and amantadine.

N *	cAMP pmoles . mg protein^{-1} Control	Dopamine (100 µM)	Apomorphine (100 µM)	Amantadine (100 µM)	Mescaline (10 µM)
(10)	15.12 ± 1.45	41.50 ± 4.21			
(8)	13.18 ± 0.81		56.29 ± 10.18		
(10)	16.60 ± 1.51			13.51 ± 1.10	
(10)	14.93 ± 1.39				15.62 ± 0.55

* Number of retinas for each experiment, half of the pool being used as control. Values are means ± S.E.M.

"dopamine-like" compounds) require an intact discriminator-transducer-amplifier complex, while the true neurotransmitter can act directly on the catalytic site of the receptor.

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