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Dopamine receptor sensitivity in brain and retina of rats during aging

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Age-related changes of neurohormonal[4], endocrine and autonomic functions have been documented[5,24] which may be the basis of diverse modes of cell functioning[3]. The unique role of the brain in adaption to environment through its integrations with the entire body makes fascinating the hypothesis that the CNS may play a key role as a pacemaker in the rate of aging[15]. There is much experimental evidence indicating that different neurochemical systems in the brain are modified during aging[15]. Striatal dopamine metabolism is affected[17], the catabolism of norepinephrine in hypothalamus is slowed[3]; dopamine uptake by hypothalamic and striatal synaptosomes appears to be reduced[7]; the sensititivty of adenylate cyclase to catecholamine in cerebellum, cortex, hippocampus and caudate is reduced[27]. All these data confirm the hypothesis of a general decrease in the function of the central nervous system. In this line we have studied the relationships among aging and the dopaminergic system at the level of dopaminergic receptors.

Therefore, in view of recent studies[2,6,8,23], demonstrating in several dopaminergic areas of the CNS a dopamine-stimulated adenylate cyclase which displays many characteristics of a dopamine receptor, we have investigated the activity of dopamine-stimulated adenylate cyclase in homogenates of striatum, nucleus accumbens, tuberculum olfactorium, substantia nigra and retina of mature and senescent rats.

Mature (2–3-month) or senescent (20–24-month) male Sprague–Dawley rats (Charles River, Calco, Italy) were used in our experiments. Senescent animals with pathological affections (tumors, respiratory infections etc.) were excluded from the study. Mature or senescent rats were randomly caged on the same shelves to avoid environmental differences. Rats were housed at constant temperature and humidity and exposed to a light cycle of 12 h a day. Animals had free access to food and water.

The different brain areas have been dissected following the method indicated by Horn et al.[6] for nucleus accumbens and tuberculum olfactorium, and by Koslow et al.[10] for substantia nigra.

Adenylate cyclase activity was measured following the method of Kebabian et al.[8] with minor modifications. After 3 min of incubation the reaction was stopped by boiling the samples for 3 min. Cyclic AMP was purified and assayed using the method

TABLE I

Adenylate cyclase activity in different brain areas of mature and senescent rats after in vitro incubation with dopamine (DA, 5 × 10^{-6} M)

Values are expressed as pmoles of cyclic AMP formed/min/mg protein and as the mean (± S.E.M.) for triplicate determinations on each of 10–12 samples.

Brain areas	*Mature*			*Senescent*		
	Controls	*DA, 5 × 10^{-6} M*	*% Stimulation*	*Controls*	*DA, 5 × 10^{-6} M*	*% Stimulation*
Striatum	239 ± 16	486 ± 17	102	268 ± 17	370 ± 26	38
Nucleus accumbens	204 ± 14	422 ± 15	107	227 ± 16	311 ± 9	37
Substantia nigra	205 ± 11	430 ± 5	110	201 ± 6	273 ± 16	36
Tuberculum olfactorium	141 ± 9	324 ± 11	129	139 ± 4	215 ± 9	55

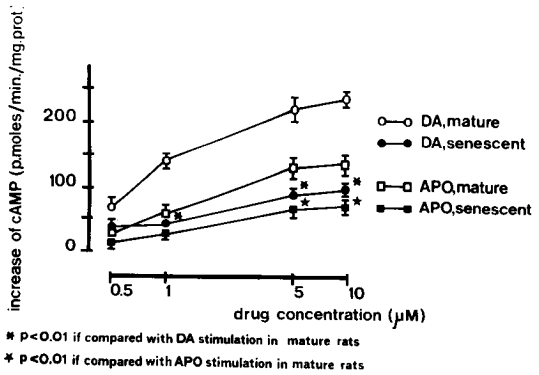

Fig. 1. Effect of various concentration of dopamine (DA) or apomorphine (APO) on adenylate cyclase in nucleus accumbens homogenates of mature and senescent rats. In the absence of drugs 216 ± 11 (mature) and 246 ± 24 (senescent) pmoles of cAMP were formed/min/mg protein. Values are expressed as the mean (± S.E.M.) for triplicate determinations on each of 10–12 samples.

of cyclic AMP-dependent protein kinase described by Kuo and Greengard[12]. Protein was measured according to Lowry et al.[13].

Table I summarizes the results of dopamine-stimulated formation of cyclic AMP in homogenates of different rat brain areas. Striatum, nucleus accumbens, tuberculum olfactorium and substantia nigra of senescent rats, with no exception, show a marked decrease of dopamine-stimulated activity of the enzyme when compared to mature rats. As an example, Fig. 1 shows concentration response curves of the dopamine-stimulated adenylate cyclase in nucleus accumbens homogenates of mature and senescent rats after adding dopamine or apomorphine.

The enzyme from senescent rats is less responsive to the stimulation of both agents. Curves with a similar shape were observed in the other brain areas examined.

On the contrary the dopamine-stimulated adenylate cyclase in retina of senescent rats shows an opposite pattern of activity. As shown in Table II, the formation of cyclic AMP induced by dopamine in retina homogenates of senescent rats is about two-fold that induced in retina homogenates of mature rats.

Apomorphine and LSD, which have been shown to exert a significant stimulatory effect on dopamine-stimulated adenylate cyclase activity in various brain areas[18]

TABLE II

Effect of dopamine (DA), apomorphine (APO) and LSD on the adenylate cyclase activity in retina homogenates of mature and senescent rats

Values are expressed as pmoles of cyclic AMP formed/min/mg of protein and as the mean ± S.E.M. for triplicate determinations on each of 10–12 samples.

	Mature	*% Stimulation*	*Senescent*	*% Stimulation*
Controls	11 ± 2	—	12 ± 1	—
DA, 10^{-5} *M*	25 ± 3	127	39 ± 1	225
APO, 10^{-5} *M*	18 ± 3	67	33 ± 3	175
LSD, 10^{-5} *M*	21 ± 2	84	31 ± 2	158

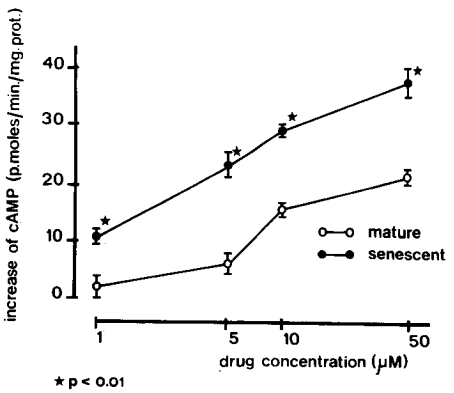

Fig. 2. Effect of various concentrations of dopamine (DA) on adenylate cylcase in retina homogenates of mature and senescent rats. In the absence of dopamine 9 ± 1 (mature) and 12 ± 2 (senescent) pmoles of cAMP were formed/min/mg protein. Values are expressed as the mean (± S.E.M.) for triplicate determinations on each of 10–12 samples.

and in the retina[19,22], were also tested. As with dopamine, also apomorphine and LSD are about two-fold active in stimulating the activity of retina adenylate cyclase of senescent rats in comparison to mature rats.

A concentration–response curve for dopamine-stimulated formation of cyclic AMP in retina homogenates of mature and senescent rats is shown in Fig. 2. The increase in the activation of adenylate cyclase observed in retina homogenates of senescent rats is higher than that observed in retina homogenates of mature rats.

Moreover, in our experimental conditions the basal activities of dopamine-sensitive adenylate cyclase of the various brain areas and retina in mature and senescent animals were comparable.

The age-related changes in the sensitivity of dopamine-stimulated adenylate cyclase in striatum, substantia nigra and dopaminergic areas of the limbic system of senescent rats are in line with previous observations on a general decline of brain function during senescence[15]. On the other hand, the reduced response of adenylate cyclase from senescent rats to dopamine and dopamine agonists may derive from a derangement of various dopaminergic mechanisms reported to be reduced in senescent rats, such as dopamine concentration and turnover, catabolism and uptake[3,7].

A special interest may arise from the observed decreased activity of the dopamine-stimulated adenylate cyclase in the substantia nigra of senescent rats. The recent localization of a dopamine-stimulated adenylate cyclase in this nucleus[9,16,21] has attracted attention to the physiological role of nigral dopamine receptors[14].

Moreover, substantia nigra is a nucleus with high vascularization, and therefore may be one of the first brain areas affected by aging.

On the other hand, the increased activity of dopamine-stimulated adenylate cyclase in the retina of senescent rats indicates that the reduction of sensitivity of this enzyme observed in the brain is not a general phenomenon. It has been reported[21,22] that dopamine-stimulated adenylate cyclase of the retina may be a tool for the investigation of receptor denervation supersensitivity[2,25,26]. Dopamine, in fact, in the

retina appears to be released as a consequence of light stimulation[11], and it therefore is not surprising that light deprivation results in a hypersensitivity of retinal dopamine-stimulated adenylate cyclase[19,22]. The enhancement of dopamine-induced stimulation of adenylate cyclase activity in retina homogenates from senescent rats may thus reflect a reduced function with aging of retinal dopaminergic neurons. It is tempting to hypothesize that this condition may result in a sort of functional denervation to which the receptor and the adenylate cyclase system react with a supersensitive response.

Just before this paper was submitted a detailed study of the rat striatal adenylate cyclase was published by Pury and Volicer (Mechanisms of Aging and Development, 6 (1977) 53-58), which also showed that dopamine-stimulated activity decreases with age.

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