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Age-related and sex-related alterations in β-adrenergic receptors in different regions of rat brain

C.S. Paulose [1] and M.S. Kanungo

Biochemistry Laboratory, Department of Zoology, Banaras Hindu University, Varanasi-221005, India

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Summary

The binding of (−)[^{3}H]dihydroalprenolol, an antagonist of norepinephrine, to β-adrenergic receptors in different regions of the brain of male and female rats of various ages was measured. The binding to the synaptosomal fraction of corpus striatum, hypothalamus, cerebral cortex, cerebellum and the brainstems shows a significant decrease in the binding in old rats of both sexes. Only in the female corpus striatal region, the binding in the adult and the old is the same. In the case of females, the highest binding is seen in the young. In the male, an increase in binding occurs up to adulthood, after which it declines, suggesting a definite sex-related difference in the β-adrenergic receptor.

β-adrenergic receptor; aging; norepinephrine; dihydroalprenolol; propranolol

Introduction

Earlier studies from this laboratory have shown that the specificity of [^{3}H]estradiol binding receptor protein of the brain of rats gradually decreases with age (Kanungo et al., 1975). Also, [^{3}H]atropine binding to muscarinic acetylcholine (Ach) receptor of cerebral and cerebellar cortices of male and female rats decreases in old age (James and Kanungo, 1976). There are a number of recent studies on age-related alterations in β-adrenergic receptor in various regions of rat brain (Greenberg and Weiss, 1978; Maggi et al., 1979; Weiss et al., 1979; Misra et al., 1980; Pittman et al., 1980). In the present study, the binding of [^{3}H]dihydroalprenolol ([^{3}H]DHA) to β-adrenergic receptors has been undertaken, in different regions of brain of male and female rats of various ages, to further clarify the age-related and sex-related alterations.

[1] *Present address*: Department of Biochemistry, Faculty of Medicine, University of Manitoba, Winnipeg, Manitoba, R3E OW3, Canada.

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Materials and methods

Albino rats of Wistar strain maintained in standard laboratory conditions were used for all experiments. Male and female rats of three age groups representing prereproductive (7 wk), reproductive (30 wk), and late reproductive (80 wk) were used. They were killed by cervical dislocation and the brain regions, corpus striatum, hypothalamus, cerebral cortex, cerebellum, and brainstem were dissected according to the method of Glowinski and Iversen (1966). The binding of [^{3}H]DHA to β-adrenergic receptors was studied in crude membrane fractions of these regions of the brain (Alexander et al., 1975). Brain regions were homogenized in cold buffer (0.25 M sucrose, 5 mM Tris-HCl, 1 mM $MgCl_2$, pH 7.4) using a Potter–Elvehjem homogenizer fitted with a Teflon pestle. The homogenate was then centrifuged for 20 min at $800 \times g$ at 4°C and the supernatant was centrifuged at $14{,}000 \times g$ for 15 min. The pellet was washed 2–3 times in sucrose buffer. The resulting pellet was then resuspended in cold buffer (75 mM Tris-HCl, 25 mM $MgCl_2$, pH 7.4) to a final concentration of 200 to 300 mg/100 μl and was used for the binding studies. Binding assays in different regions were done using a ligand concentration of 18 nM which is several times higher than the observed Kd values (4–5 nM). The nonspecific binding was found out by adding 100 μM of DL-propranolol–HCl to the incubation mixture containing 0.2 to 0.3 mg synaptosomal membrane protein and 18.0 nM (−)[^{3}H]DHA. Specific [^{3}H]DHA binding was obtained by subtracting the amount not displaced by unlabelled propranolol–HCl. Protein of the crude membrane fraction was determined (Lowry et al., 1951) and the specific binding was expressed as pmol of [^{3}H]DHA bound/mg protein. Each set of data was collected from 4 to 6 rats, and statistically analyzed.

Results and Discussion

The binding of [^{3}H]DHA to the synaptosomal of all five regions of the brain is significantly lower in old rats of both sexes (Tables I and II). These findings confirm and extend the previous studies (Greenberg and Weiss, 1978; Maggi et al., 1979 and Misra et al., 1980), where they have reported a decrease in β-adrenergic receptor binding in cerebellum, brainstem and cerebral cortex. Only in the female corpus striatal region, the binding in the adult and the old is the same. A sex difference in [^{3}H]DHA binding is also noticed. In females, the highest binding is seen in the young and the binding decreases with increasing age. In the males, the binding increases up to adulthood after which it declines. Hydrocortisone, deoxycorticosterone, 17β-estradiol, and progesterone have all been shown in vitro to enhance catecholamine sensitivity of the aorta resulting in enhanced vasoconstriction which could raise arterial blood pressure (Besse and Bass, 1966; Kalsner, 1969). Krall et al. (1978) noted that in rats, estrogen treatment reduced the β-adrenergic receptor number. Further, castration induced a rise in uterine β-adrenergic receptor number. The above reports clearly show that sex steroid levels alter the receptor levels. From our results (Tables I and II) a difference in the β-adrenergic receptor numbers in

TABLE I

Specific (−)[^{3}H]dihydroalprenolol binding (pmol/mg protein) to synaptosomal fractions of corpus striatum (CS), hypothalmus (HYPO), cerebral cortex (CC), cerebellum (CB), and brainstem (BS) of female rats of various ages.

Brain regions	7 wk	30 wk		80 wk	
	Mean SEM	Mean SEM	*P*	Mean SEM	*P*
CS	0.162± 0.040	0.041± 0.004 (−75%)	0.05	0.051±0.004 (+25%)	NS
HYPO	0.140± 0.009	0.112± 0.012 (−21%)	NS	0.041± 0.002 (−64%)	0.001
CC	0.102± 0.016	0.031± 0.005 (−70%)	0.01	0.021± 0.003 (−33%)	NS
CB	0.060± 0.003	0.050± 0.003 (−17%)	0.05	0.020± 0.002 (−60%)	0.001
BS	0.042± 0.002	0.041± 0.001	NS	0.031± 0.002 (−25%)	0.01

The mean value for each group is calculated from 4 to 6 animals.
SEM=standard error of the mean; *P*=level of significance; −=inhibition; +=stimulation; NS=not significant.

males and females at various ages may be seen. This may account, at least partly, to the behavioral differences of the two sexes. A regional binding of [^{3}H]DHA is also seen which may be due to differences in the rate and time of the maturation process that occurs in different regions of the brain.

TABLE II

Specific (−)[^{3}H]dihydroalprenolol binding (pmol/mg protein) to synaptosomal fractions of corpus striatum (CS), hypothalamus (HYPO), cerebral cortex (CC), cerebellum (CB), and brainstem (BS) of male rats of various ages.

Brain regions	7 wk	30 wk		80 wk	
	Mean SEM	Mean SEM	*P*	Mean SEM	*P*
CS	0.071± 0.003	0.102± 0.008 (+43%)	0.01	0.021± 0.004 (−80%)	0.001
HYPO	0.051± 0.003	0.102± 0.009 (+100%)	0.01	0.020± 0.002 (−80%)	0.001
CC	0.070± 0.003	0.160± 0.014 (+128%)	0.001	0.020± 0.001 (−88%)	0.001
CB	0.071± 0.002	0.102± 0.007 (+43%)	0.01	0.020± 0.003 (−80%)	0.001
BS	0.061± 0.006	0.173± 0.015 (+183%)	0.001	0.010±0.002 (−94%)	0.001

The mean value for each group is calculated from 4 to 6 animals.
SEM=standard error of the mean; *P*=level of significance; −=inhibition; +=stimulation; NS=not significant.

The decrease in β-adrenergic receptors in the old may be due to the destruction of synapses and/or loss of neurons which is reported to occur in old age (Johnson and Erner, 1972). Since the functions of brain are mediated through neurotransmitters that act on specific receptor sites, the decrease in receptor proteins in old age may contribute to impairment of brain function in old age. This is consistent with the model for aging proposed by Kanungo (1975, 1980).

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