AGING PROCESS DECREASES THE DENSITY OF MUSCARINIC RECEPTORS IN RAT ADENOHYPOPHYSIS

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1. Introduction

Several histochemical studies report neuronal loss and reduction in the number of dendrites in various brain regions of aged men and animals which can be correlated with the decrease in activity of different neuronal populations during aging [1,2]. The strong correlation between neurotransmitter receptor function and the ability to evoke a physiological event raises the possibility that some behavioral and physiological patterns characteristic of aging are due to changes in synaptic biochemical mechanisms.

The pituitary gland is an obvious candidate as a regulator of aging because of the key role it plays in the control of most other endocrine glands as well as in regulating the processes of growth, reproduction and metabolism [3]. In aged female rats, for example, the regular estrous cycle ceases, reflecting an ovarian disturbance which is thought to be secondary to dysfunction of the hypothalamic -pituitary axis [4]. Our data has indicated the presence of muscarinic receptors in both the hypothalamus and the adenohypophysis of rats, and the participation of the muscarinic cholinergic system in the modulation of gonadotropin release [5–7]. This investigation was done to test the hypothesis that the number of muscarinic receptors in the pituitary–hypothalamus axis, or their affinity, or both, diminish with advancing age, and that this may account for some of the observed physiological phenomena associated with aging.

2. Experimental

2.1. *Materials*

N-[^{3}H]Methyl-4-piperidylbenzilate (*N*[^{3}H]M4PB) (33 Ci/mmol) and unlabeled muscarinic agonist oxotremorine were prepared as in [6]. Adult and aged female rats of the CD strain were supplied by Levinstein's farm (Yokneam) and maintained in an airconditioned room at 24 ± 2°C for 14 h daily. Food (Assia Maabarot Ltd, Israel) and water were supplied ad libitum. After an adjustment period of at least 4 weeks, daily vaginal smears were taken of all mature females, and only those having a regular 4-day estrous cycle were used. The mature rats were then 3–4 months old and weighed 190–250 g. Aged females were 20 months old, weighed 290–350 g, and vaginal smears showed persistent estrous. These rats have a maximum life span of 30–35 months, with 50% mortality at ~25–28 months.

2.2. *Binding assays*

Full details concerning homogenate preparations are in [5,6]. Direct binding of *N*-[^{3}H]methyl-4-piperidyl benzilate to muscarinic receptors, as well as competition experiments with agonists, in homogenates of pituitary, median and posterior hypothalamus, preoptic area, medulla-pons and cortex of female rats, were done as in [5,6,8]. Specific binding is defined as the total binding at 25°C and pH 7.3 of *N*[^{3}H]M4PB to 50 μl tissue preparation (3% homogenate, w/v) in 2 ml buffer (except for the pituitary, in which the total volume was 0.6 ml) minus the non-specific binding, i.e., binding in the presence of 1 μM of unlabeled atropine.

2.3. *Data analysis*

The data obtained from direct binding assays with the antagonist were analyzed by a non-linear, least-square, curve-fitting procedure using a generalized model for complex ligand–receptor systems [6].

Theoretical competition curves were fitted to the

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Table 1
The binding characteristics of *N*-[^{3}H]methyl-4-piperidylbenzilate in the various brain regions of the female rat[a]

Brain region	Mature		Aged	
	K_d (nM)	B_{max} (pmol/mg protein)	K_d (nM)	B_{max} (pmol/mg protein)
Cortex	0.41 ± 0.03	0.91 ± 0.06	0.45 ± 0.02	0.90 ± 0.1
Medulla	0.82 ± 0.02	0.29 ± 0.05	0.71 ± 0.09	0.33 ± 0.05
Median hypothalamus	0.54 ± 0.04	0.38 ± 0.04	0.50 ± 0.02	0.35 ± 0.05
Posterior hypothalamus	0.62 ± 0.02	0.58 ± 0.01	0.80 ± 0.01	0.54 ± 0.08
Preoptic area	0.80 ± 0.03	0.40 ± 0.05	0.85 ± 0.08	0.50 ± 0.07

[a] The binding parameters ±SD are the average values of ⩾3 expt each performed in triplicate as detailed in [5,8]

experimental data points using the non-linear, least-square, regression computer program BMDPAR (November 1978 revision), as detailed in [7–9]. (The program was developed at the Health Science Computing Facility of the University of California, Los Angeles; the Facility is sponsored by NIH Special Research Resources Grant RR-3.)

3. Results and discussion

As shown in table 1, the binding characteristics (K_d and B_{max}) of the highly specific muscarinic antagonist *N*[^{3}H]M4PB in the various brain regions of aged female rats were similar to those in mature rats. On the other hand, in the adenohypophyses of aged rats significant changes were observed (table 2). Previous data from our laboratory have indicated that the binding curves of [^{3}H]antagonists in adenohypophysis are best explained by assuming the existence of two subclasses of affinity sites [6], i.e., high (α-) and low (β-) affinity sites. As shown in table 2, while the dissociation constants (K_d) for the two sites are similar in mature and aged rats, maximal binding capacity (B_{max}) in the aged rats is lower by 29% for the high affinity sites and ~50% for the low affinity sites. The estrous stage was chosen for comparison, since all the aged females exhibited persisting estrous characteristics in vaginal smears. A further difference between the aged and the mature females was seen in agonist binding. Assuming the existence of two binding states [high (H) and low (L)], as detailed in [9,10], an analysis of the competition curves obtained with adenohypophyseal homogenates using oxotremorine as the agonist ligand reveals no change in either K_H or K_L (in both mature and aged rats), but a decrease in the proportion of high affinity binding sites: 57% (in mature females) to 21% (in aged females) (fig.1). As seen in fig.1 there was no change with aging in the proportions of either the low or the high affinity binding sites in cortex, medulla, median or posterior hypothalamus, or preoptic area. The values of K_H for these regions are (nM): 6 ± 1; 15 ± 1.5; 4.5 ± 0.5; 11 ± 1.8; 27 ± 2.5 for mature rats as compared with 5 ± 2.5; 11 ± 2; 7 ± 1; 24 ± 2; 23 ± 3 for aged rats,

Table 2
N-[^{3}H]Methyl-4-piperidylbenzilate binding characteristics in adenohypophysis of female rats[a]

	Mature[b]	Aged
K_α(nM)	0.64 ± 0.06	0.8 ± 0.1
B^α_{max} (fmol/mg protein)	65 ± 5	46 ± 2[c]
K_β(nM)	11.2 ± 0.9	9.5 ± 1.7
B^β_{max} (fmol/mg protein)	157 ± 10	83 ± 1[c]

[a] α and β designate the higher and lower affinity antagonist binding sites, respectively, as described in the text. The binding parameters ±SD are the average values of ⩾3 expt each performed in triplicate as detailed in [6]
[b] Females at the estrous stage; [c] $p < 0.001$ of aged *vs* mature

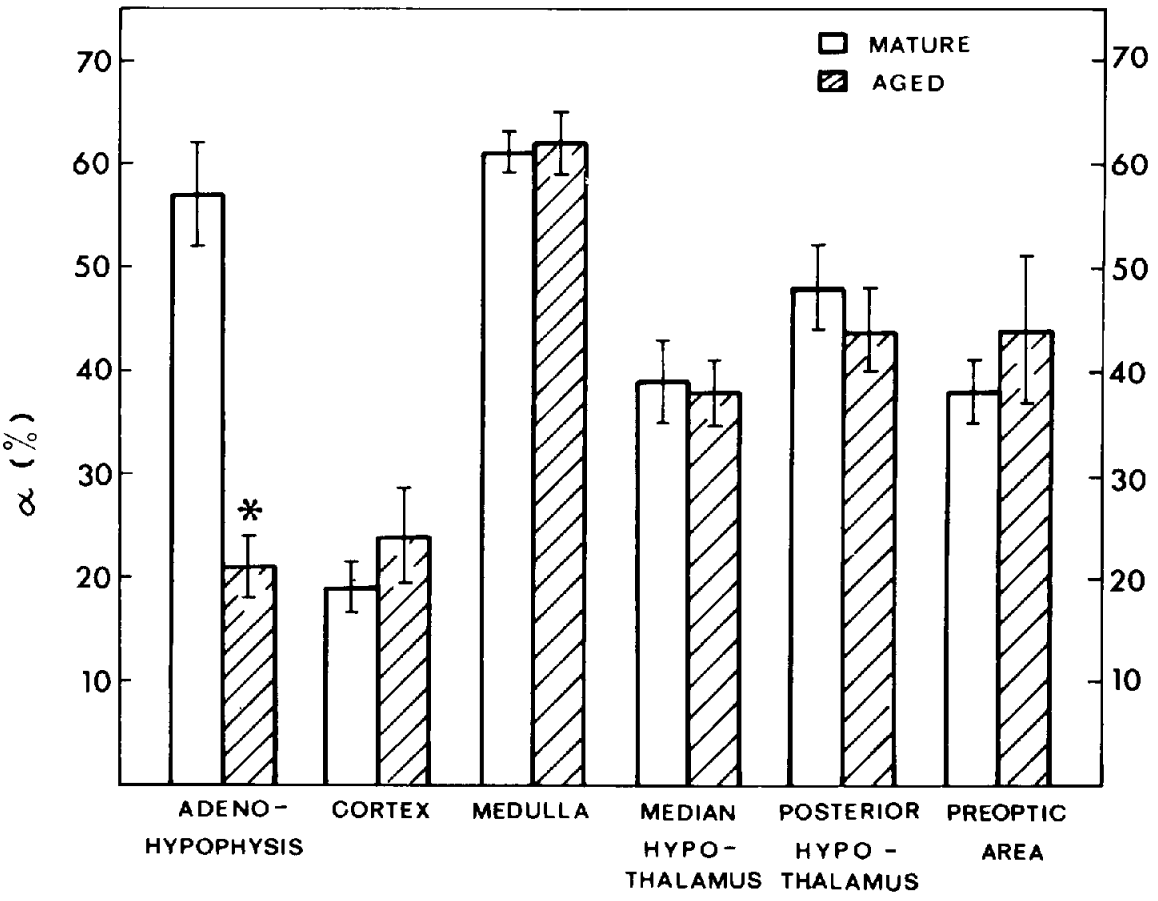

Fig.1. Proportion of high affinity binding sites (α) (see text) for the muscarinic agonist oxotremorine in the various homogenates under investigation. Values ±SD were calculated by the non-linear, least-square, regression procedure for a two-site model as detailed in [5,6,9]; * $p < 0.001$ of aged *vs* mature

respectively. The indirect measurement of agonist binding, by competition experiments precludes an absolute estimation of the number of agonist binding sites, since the only observation possible is that all $N[^3H]$M4PB binding sites can be blocked by oxotremorine whereas the extent to which oxotremorine is blocked by $N[^3H]$M4PB cannot yet be directly assessed. The changes in proportion of high and low agonist affinity sites given in fig.1 are from 57% and 43% (in mature rats) to 21% and 79% (in aged rats), respectively. The latter values were obtained assuming a value for the antagonist binding site which is 59% of that for mature rats. Hence, normalizing the values of agonist binding to 100% yields a value of 12% for high affinity sites and 48% for low affinity sites, thus demonstrating that the effect of age is exerted only on the high affinity sites, i.e., it results in their decrease from 57- 12%.

A loss of muscarinic receptor(s) in whole brains of aging mice [11] led to the conclusion that the observed decrease in ligand binding was not the result of neuron death [11]. It therefore seems that other changes in nerve cells and nerve processes must be responsible for functional aging. Losses in other receptor molecules in aging rodents have also been reported, e.g., dopamine receptors in the striatum [12], β-adrenergic receptors in the pineal gland [13] and cytosol receptors for steroid sex hormones in the hypothalamus [14].

Surprisingly, loss in muscarinic receptors has not been observed in this study in the hypothalamus, which is known to regulate adenohypopyseal func-

tion. Our results, however, do not exclude the possibility that changes might occur in other parameters, e.g., acetylcholine content or turnover rate, which affect the functioning of the cholinergic system in the hypothalamus, and that these changes might account in part for the impaired functioning of the hypotalamus in aging rats [4].

In [7] muscarinic agonist binding properties in the rat adenohypophysis were affected by steroid sex hormones (β-estradiol and progesterone), both of which resulted in a decrease in the proportion of high affinity agonist binding sites [7]. These findings, suggest a link between the muscarinic system and the mechanism by which these steroids exert their gonadotropin-releasing effect on the adenohypophysis. According to this hypothesis [7] a decrease in muscarinic activity, i.e., in binding capacity, will be accompanied by a reduction in uptake of steroid sex hormones via their respective transport system (receptors). The results presented here are compatible with this hypothesis, which is further reinforced by:

(i) The ability of aging females to exhibit a positive feedback via estrogen and progesterone on LH release is significantly depressed although not completely eliminated [15];

(ii) A decrease in estrogen uptake by the pituitaries of aged female rats [16].

These results support the notion that the molecular basis of the aging process is to be found, at least in part, in a loss of receptor molecules for neurotransmitters and hormones.

References

[1] Buell, S. J. and Coleman, P. D. (1979) Science 206, 854–856.
[2] Feldman, M. L. (1976) Aging 3, 211–213.
[3] Everitt, A. V. and Burgess, J. A. (1976) in: Hypothalamus, Pituitary and Aging (Everitt, A. V and Burgess, J. A. eds) pp. 3–4, Charles C. Thomas, Springfield IL.
[4] Aschheim, P. (1976) in: Hypothalamus, Pituitary and Aging (Everitt, A. V. and Burgess, J. A. eds) pp. 376–418, Charles C. Thomas, Springfield IL.
[5] Avissar, S., Egozi, Y. and Sokolovsky, M. (1981) Neuroendocrinology 32, 295–302.
[6] Avissar, S., Egozi, Y. and Sokolovsky, M. (1981) Neuroendocrinology 32, 303–311.
[7] Sokolovsky, M., Egozi, Y. and Avissar, S. (1981) Proc. Natl. Acad. Sci. USA in press.
[8] Kloog, Y., Egozi, Y. and Sokolovsky, M. (1979) Mol. Pharmacol. 15, 545–558.
[9] Kloog, Y., Michaelson, D. M. and Sokolovsky, M. (1980) Brain Res. 194, 97–117.
[10] Birdsall, N. B. M., Burgen, A. S. V. and Hulme, E. C. (1978) Mol. Pharmacol. 14, 723–736.
[11] Freund, G. (1980) Life Sci. 26, 371–375.
[12] Memo, M., Lucchi, L., Spano, P. F. and Trabucchi, M. (1980) Brain Res. 202, 488–492.
[13] Greenberg, L. H. and Weiss, B. (1978) Science 201, 61–63.
[14] Masafumi, H., Kato, K. I., Nawata, H. and Ibayashi, H. (1981) Brain Res. 204, 373–386.
[15] Meites, J., Huang, H. H. and Simpkins, J. W. (1978) in: The Aging Reproductive System (Schneider, E. L. ed) pp. 213–235, Raven Press, New York.
[16] Peng, M. T. and Peng, Y. M. (1973) Fert. Steril. 24, 534–539.