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BRIEF COMMUNICATION

Kinetics of [^{3}H]-Prazosin Binding to the Rat Cortex During Aging

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NOWAK, G. AND J. SILBERRING. *Kinetics of [^{3}H]-prazosin binding to the rat cortex during aging.* PHARMACOL BIOCHEM BEHAV 31(2) 505–507, 1988.—Kinetics (B_{max}, K_D, k_{on}, k_{off}) of [^{3}H]-prazosin binding to the rat cortex was measured at different ages of animals (2–4, 13–15, 20–24 months old). Additionally, an agonist (phenylephrine) and an antagonist (phentolamine) inhibition of [^{3}H]-prazosin binding was performed at two different rat ages (2–4 and 20–24 months old). The number of cortical α_1-adrenoceptors was significantly reduced in 20–24-month-old rats, when compared with 2–4-month ones. Association (k_{on}) and dissociation (k_{off}) rate constants were also reduced in the oldest group, but the dissociation constant (K_D) was similar in all the age groups tested. Affinity of phenylephrine, but not of phentolamine, was reduced in the oldest group (20–24 months old). The obtained data suggest that changes in the brain α_1-adrenoceptors, correlated with the animal age, are connected to the number of binding sites, rate constants and the affinity of an agonist.

[^{3}H]-Prazosin Receptor binding Kinetics Rat cortex Aging

A decrease in the density and turnover of brain catecholaminergic receptors with age was reported previously [e.g., (3, 5, 6)]. However, the literature data concerning the influence of aging on α_1-adrenoceptors in the rat brain are still disputable. DeBlasi *et al.* (2) found no changes in [^{3}H]-WB-4101 binding to the cortex of 2- and 21–23-month-old rats, but Misra *et al.* (7) found that the specific binding of [^{3}H]-WB-4101 to rat cortical membranes declined by approximately 35% between the 5th and 25th month of age. Greenberg and Weiss (4), who used a more specific α_1-adrenoceptor antagonist, [^{3}H]-prazosin, reported a loss of the rat cortical α_1-adrenoceptors during aging.

Our previous study (10) reported that the [^{3}H]-prazosin binding to the cortex of 2–4- and 11–12-month-old rats is similar.

The aim of the present study was to assess the [^{3}H]-prazosin binding as regards intrinsic kinetic parameters measured in a wider age range.

METHOD

Male Wistar rats, 2–4 months old, weighing 180–220 g; 13–15 months old, weighing 550–600 g; and 20–24 months old, weighing 500–600 g, were housed in groups of 10 (2–4 months old) or 5 (13–15 and 20–24 months old) animals per cage, under standard laboratory conditions (constant 12 hr light/dark cycle, light at 6.00–18.00 hr, food and water ad lib). The experiments were carried out during the summer season.

The rats were killed by decapitation between 10.00–12.00 hr. Their brains were removed and the neocortex was dissected and rapidly frozen on dry ice. The radioligand used for the binding to α_1-adrenoceptors was [^{3}H]-prazosin. The preparation of homogenates and the assay procedure were identical with those described previously (10), except for the preparation of nonspecific binding samples that were defined in the presence of 4 μM phentolamine rather than 1 μM phentolamine.

Six increasing concentrations of [^{3}H]-prazosin, ranging from 0.05 to 1.5 nM, were used for the saturation experiments. Values of B_{max} and K_D were calculated individually for each rat by the Scatchard analysis (15), and the points were assayed in duplicates. The inhibition of binding by cold ligands [8 concentrations in triplicates: phentolamine (10^{-10}–10^{-4} M) and phenylephrine (10^{-8}–10^{-3} M)] was calculated from the regression of log percentage inhibition of specific binding on log concentration of cold displacer. The inhibition constant K_i was calculated from the Cheng-Prusoff formula (1).

The association rate constant was calculated by the initial velocities method according to Rodbard (13). The dissociation experiment was initiated by the addition of an "infinite" concentration of cold phentolamine (4 μM) after the equilibrium was reached.

The analysis of variance, followed by Student's *t*-test, was applied to determine the statistical significance of the obtained data (protected lowest significant difference test for the data in Table 1). An unweighted linear regression was used for the evaluation of binding data from the Scatchard plot. Kinetic curves were analyzed with the aid of nonlinear curve-fitting iterative program containing the Nelder and Mead optimization algorithm and based on a simplex method (8).

TABLE 1
[^{3}H]-PRAZOSIN BINDING TO RAT NEOCORTICAL MEMBRANES

	Age (months)		
	2–4	13–15	20–24
B_{max} (pmol/g tissue)	6.82 ± 0.49	5.86 ± 0.47	4.62 ± 0.63*
K_D (nM)	0.13 ± 0.020	0.15 ± 0.030	0.11 ± 0.015

*$p<0.05$ vs. a group of 2–4-month-old rats.
Results are expressed as a mean ± SEM of 4–5 Scatchard plots.

All the reagents used were purchased from commerical sources.

RESULTS AND DISCUSSION

In the present paper we obtained data that support the hypothesis that rat brain α_1-adrenoceptors exhibit changes in physico-chemical parameters during aging [see (3) for review]. The results obtained from the binding of [^{3}H]-prazosin to the rat brain neocortex are presented in Table 1. As can be seen, a significant fall (by 32%) in B_{max} was observed in 20–24-month-old rats, but not in 13–15-month ones (a non-significant 14% fall). Moreover, there were no changes in K_D values in either group. The binding of a labeled ligand was a saturable and reversible reaction for which the Scatchard analysis revealed one binding site (also confirmed by the Hill coefficient—data not shown). A steady state of the ligand-receptor interaction was reached after 20 min at 25°C (young rats), and after 25 min for 20–24-month-old rats (Fig. 1). The above discrepancies are also reflected in the association rate constant (k_{on}), which was 1.7 times higher in the younger rats than in 20–24-month-old ones ($p<0.05$) (Table 2). The dissociation rate constant (k_{off}) was calculated two ways in order to obtain biased values. The parameters calculated from the decay curve were about twice as high as in the oldest rats, while those obtained with the aid of K_D differed by a factor of 1.5 ($p\leq0.05$). We feel that the more accurate values for k_{off} are those obtained from the dissociation experiment, since the total error in k_{off} was lower than that cummulated during determination of K_D and k_{on}. We also calculated K_D parameters using the obtained values of the rate constants. In comparison with K_D obtained from the Scatchard plots, both values are of the same order (Tables 1 and 2). The decreasing association and dissociation rate constants of ligands (rate of ligand-receptor combination) during the process of aging, suggest impairment of the neurotransmission at the receptor level [according to (11)]. The inhibition constants (K_i) of phentolamine and phenylephrine (Table 3) were similar to those obtained by Reader *et al.* (12). However, K_i parameters indicate different changes during aging. When the K_i value of phentolamine (antagonist) is similar in all the age groups tested, the K_i one of phenylephrine (agonist) is significantly higher (by about 80%) in 20–24-month-old rats than in their 2–4-month-old counterparts (Table 3). This finding suggests that an agonist rather than antagonist affinity is diminished in the aged rat brain.

Zhou *et al.* (16) found that the synthesis rate of α_1-adrenoceptors in the aged rat cortex is decreased; this finding is in agreement with our results (9) which show the inability of cortical α_1-adrenoceptors in elderly rats to increase their density after an antidepressant treatment.

Our studies have put forward a suggestion that changes in brain α_1-adrenoceptors correlated with the animal age are connected with the number of binding sites, rate constants and the affinity of the agonist rather than the antagonist. In consequence, this observation may suggest a diminished postreceptor action in the brain of aged rats [see (14) for review].

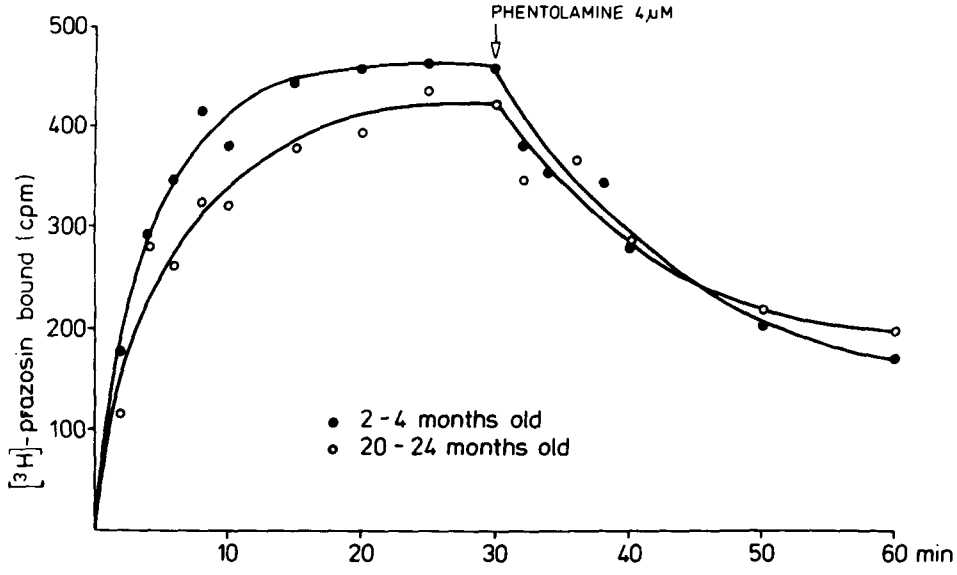

FIG. 1. Association and dissociation of [^{3}H]-prazosin: solid circles—2–4-month-old rat neocortical membranes, open circles—20–24-month-old rat neocortical membranes. [^{3}H]-Prazosin (0.1 nM) was incubated with neocortical membranes at 25°C at different time intervals. Each point represents a mean value of duplicate determinations. After a 30-min incubation, the specific binding was measured at different times after addition of phentolamine (4 μM).

TABLE 2

KINETIC PARAMETERS OF [^{3}H]-PRAZOSIN BINDING TO RAT NEOCORTICAL MEMBRANES

Age (months)	2–4	20–24
k_{on} ($M^{-1} \cdot min^{-1}$)	0.453×10^9	0.269×10^9
k_{off} (min^{-1})		
I	0.0589	0.0300
II	0.0327	0.0220
K_D (nM) (calculated from the rate constants)	0.07	0.08

I k_{off} calculated from the equation $k_{off} = K_D \times k_{on}$.
K_D: dissociation constant calculated from the Scatchard plots.
II k_{off} calculated from the equation

$$k_{off} = \frac{\ln_{10}}{t_2 - t_1} \times \lg \frac{B_1}{B_2}.$$

B_1: amount of [^{3}H]-prazosin bound at time t_1.
B_2: amount of [^{3}H]-prazosin bound at time t_2.
The values (means) were calculated from the kinetic experiment (Fig. 1).

TABLE 3

INHIBITION CONSTANTS (K_i) OF PHENTOLAMINE AND PHENYLEPHRINE FOR [^{3}H]-PRAZOSIN (0.1–0.2 nM) BINDING TO RAT NEOCORTICAL MEMBRANES

Age (months)	Phentolamine K_i (nM)	Phenylephrine K_i (μM)
2–4	8.6 ± 2.1	3.0 ± 0.35
13–15	8.6 ± 1.3	—
20–24	7.3 ± 2.8	5.4 ± 0.42*

*$p < 0.05$ vs. a group of 2–4-month-old rats.
Results are expressed as a mean ± SEM of 2 separate experiments.

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