BRIEF REPORTS

Age-Related Changes in Peripheral Benzodiazepine Receptors of Human Platelets

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Using ^{3}H-PK 11195 as radioligand, the number and affinity of peripheral benzodiazepine receptors in platelets of 15 elderly healthy subjects were compared to those of 15 young subjects. The results showed that the dissociation constant (K_d) was significantly higher in the elderly than in the young subjects, while the density of binding sites did not differ. These findings suggest that the age-related changes in peripheral benzodiazepine receptors may be coupled with secondary changes in their hypothesized functions.

Key Words: peripheral benzodiazepine receptors, human platelets, aging

INTRODUCTION

Different types of high-affinity binding sites for benzodiazepines have been described in the central nervous system and in peripheral organs on the basis of their different affinity for radiolabeled ligands (Olsen and Venter 1986). The central receptors are mainly labeled by flumazenil (Ro 15-1788), whereas the peripheral type of benzodiazepine receptors are identified specifically by Ro 5-4864 and PK 11195 (Marangos et al 1982).

The most abundant sources of peripheral benzodiazepine receptors are kidneys, lungs, ovaries, testes and adrenal glands, but they are also present in the central nervous system, in particular in glial cells (Anholt et al 1984). They are currently attracting much interest because of the absence of coupling with the $GABA_A$ receptor-choride channel complex, and because of their specific location on the outer membrane of mitochondria, which would suggest a possible role in metabolic functions. In fact, although the physiological function of peripheral benzodiazepine receptors is still unknown, some data indicate an involvement in the synthesis of steroid hormones (Anholt et al 1986).

Animal data show that peripheral benzodiazepine receptors are modulated by stress (Drugan et al 1986) or drugs (Weizman et al 1987). There is also a growing literature on changes in some psychiatric disorders, such as generalized anxiety disorder, obsessive-compulsive disorder and Alzheimer's disease (Bidder et al 1990; Rocca et al 1991). However, no information is available on the possible changes of these receptors in elderly humans. Therefore, we evaluated the peripheral benzodiazepine receptors in the platelets of young and elderly subjects.

SUBJECTS AND METHODS

Subjects

Fifteen elderly subjects (ten female and five male, between 66 and 91 years of age; mean ± SD: 82.0 ± 9.3) and 15 young subjects (eight female and seven male, between 22

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Table 1

Demographic data and ^{3}H-PK 11195 binding parameters (B_{max} and K_d) in elderly and young subjects

	Elderly subjects				Young subjects		
	Age	B_{max}	Kd		Age	B_{max}	Kd
F	66	3180	4.07	F	30	1902	3.24
M	85	3982	5.79	M	26	3271	3.88
F	84	3433	5.37	F	27	5101	2.62
F	86	6104	4.92	M	32	3476	3.49
F	91	1217	5.60	F	22	3456	2.03
F	90	4170	4.15	F	31	1643	4.97
M	88	2651	5.29	M	33	4362	3.04
F	86	5412	5.12	F	28	7605	3.37
M	66	5929	5.05	M	36	2017	2.03
M	68	1574	5.23	M	25	3653	3.36
F	88	5548	6.41	F	28	4220	3.90
F	73	5634	3.69	M	28	1827	4.77
F	91	5258	5.55	F	28	3437	1.36
M	78	1797	5.92	M	23	2116	1.23
F	90	3633	4.69	F	26	7208	2.20
Mean ±	82.0	3968	5.12	Mean ±	28.2	3686	3.03
SD	9.3	1653	0.74	SD	3.7	1830	1.12

Age = years; B_{max} = fmol/mg protein; K_d = nM

and 36 years of age; mean ± SD: 28.2 ± 3.7) were studied (see Table 1).

None of the subjects was suffering from any physical illness, as documented by medical check-up and common laboratory tests and none of the subjects was suffering from any major psychiatric disorder, as assessed by a detailed psychiatric interview. In addition, all of the subjects were drug-free. Informed consent was obtained from all subjects.

Methods

Thirty ml of venous blood was drawn from fasting subjects between 8:00 am and 10:00 am, collected into plastic tubes containing 5 ml of anticoagulant (2.2% sodium citrate and 1.2 % citric acid) and centrifuged at 150 × g for 15 minutes at 23°C. Platelet-rich plasma was collected and centrifuged at 1500 × g for 15 minutes at 23°C. The drained platelet pellet was frozen at – 80°C until the binding assay, which was carried out within two weeks. Prior to the binding assay, the platelets were lysed and homogenized in 12 ml buffer (50 mM Tris HCl, pH 7.4) using an Ultra-Turrax homogenizer for ten seconds at 2/3 full speed and then centrifuged at 49000 × g for 15 minutes at 4°C. This procedure was immediately repeated. The membranes were re-suspended in 12 ml of 50 mM Tris HCl and used for binding studies. The binding of ^{3}H-PK 11195 to platelet membranes was performed according to a slightly modified method of Gavish et al (1986). In brief, 400 µl of membrane suspension (50 µg protein) was incubated at 0°C with 20 µl of [^{3}H]-PK 11195 (specific activity: 83.7 Ci/mmol NEN, England) at final concentrations ranging between 1 nM and 8 nM, and 50 mM Tris HCl buffer, pH 7.4, in a total volume of 500 µl. Non-specific binding was determined in the presence of 10 µM unlabeled PK 11195 (Sigma).

The incubation lasted for 90 minutes and was halted by the addition of 5 ml of ice-cold incubation buffer and filtration under vacuum over Whatman GF/B filters. The filters were washed three times with 5 ml of the same buffer, placed in vials and counted in 4 ml of scintillation cocktail in a liquid scintillation counter (Beckman).

The data were evaluated by Scatchard analysis to obtain the maximal number of binding sites (B_{max}, fmol/mg protein) and their affinity to [^{3}H]-PK 11195 expressed by the equilibrium dissociation constant (K_d, nM), by means of microcomputer programs (EBDA and LIGAND).

Protein concentration was determined according to the method of Peterson (1977). The significance of the difference

between the two groups was determined by the student *t*-test (unpaired, two-tailed).

RESULTS

The results showed that the B_{max} of [^{3}H]-PK 11195 fmol/mg protein was 3968 ± 1653 in the elderly subjects and 3686 ± 1830 in the young subjects, with no significant difference between the two groups. On the other hand, the K_d was statistically higher in the elderly (5.12 ± 0.74 *versus* 3.403 ± 1.12) than in the young subjects ($p < 0.015$) (see Table 1).

DISCUSSION

The present study shows that peripheral benzodiazepine receptors in human platelets undergo age-related changes in the sense that the dissociation constant (K_d) is higher in elderly than in young subjects. To our knowledge, this is the first observation of physiological changes of peripheral benzodiazepine receptors in elderly humans. Previously, a circadian rhythm had been described in platelet benzodiazepine receptors of humans (Levi et al 1986).

The peripheral benzodiazepine receptor appears to be sensitive to stress (Drugan et al 1986) and drugs (Weizman et al 1987). In addition, it has been described as being abnormal in some anxiety disorders (Rocca et al 1991), and the observed abnormalities involved the number of binding sites. The change in K_d reported in our study seems to be a specific phenomenon linked with age. In fact, a study (Pedigo et al 1981) reported a decreased binding of Ro 5-4864 in kidney membranes of senescent rats. Several hypotheses might explain our findings. The increase in K_d in aged subjects might result from a general membrane alteration (Zubenko et al 1987) resulting from lipid peroxidation or changes in fluidity typical of advanced age (Balin et al 1982) and also involving mitocondrial membrane where the receptor is located. Alternatively, the danger might be the result of a specific mitochondrial dysfunction linked with age (Fleming et al 1982; Marcus et al 1982; Cutler et al 1984) and may thus constitute a marker for mitochondria; in this case, similar changes should be detectable in primary inherited mitochondrial diseases. The consequences of such age-related changes might be relevant, if they occur in other cells, in particular when considering the hypothesized function of the receptor in steroidogenesis (Hu et al 1989; Jung-Testas et al 1989). In fact, it has been demonstrated that the peripheral benzodiazepine receptor in adrenals mediates the translocation of cholesterol from the outer to the inner mitocondrial membrane and it is also involved in the side chain cleavage of cholesterol (Mukhin et al 1989; Papadopoulos et al 1990). The peripheral benzodiazepine receptor may subserve the same function in other tissues, in particular in glial cells which have been shown to be able to synthetize steroids, such as pregnenolone, progesterone and its derivatives (Hu et al 1989; Jung Testas et al 1989), that strongly modulate the $GABA_A$ receptor complex and thereby the stress response (Purdy et al 1990).

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