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Short communication

Desipramine treatment differently down-regulates β-adrenoceptors of freshly isolated neurons and astrocytes

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Abstract

Eight days' desipramine administration (16 mg/kg per day i.p.) to rats resulted in a significant decrease in the density of β-adrenoceptors in neuronal and astroglial cells from rat forebrain and cerebellum without modification of their corresponding affinity. β-Adrenoceptor subtypes, β_1 and β_2, which coexist in neurons and astrocytes, are differently distributed in the brain and differently modified by desipramine administration which down-regulates β_1-adrenoceptor in forebrain neurons and astrocytes and β_2-adrenoceptor in cerebellum neurons. This down-regulation affects the predominant subtype, β_1 or β_2, of the relevant structure. Astroglial and neuronal β-adrenoceptors are differently coupled to G-proteins. Only neuronal cells contain the high-affinity conformational state of the β-adrenoceptor which is sensitive to GTP. The percentage of neuronal receptors in the high-affinity state differs according to brain area. Desipramine treatment decreases the neuronal density of both cerebellar high- and low-affinity sites and only the forebrain high-affinity site. The desipramine effects are thus subtype-dependent and differ between the two brain areas selected.

Keywords: β-Adrenoceptor, β_1 and β_2 subtype; Desipramine; Binding site, high and low affinity; Astrocyte, isolated; Neuron, isolated

1. Introduction

The tricyclic antidepressant, desipramine, is a potent norepinephrine uptake inhibitor (Banerjee et al., 1977). Its long-term administration to rats results in down-regulation of β-adrenoceptors (Argenti and D'Mello, 1994; McManus and Greenshaw, 1991). However, the different brain regions are not affected equally by desipramine (Ordway et al., 1988). β_1- and β_2-adrenoceptor subtypes are also differently down-regulated after chronic desipramine administration (Stahl et al., 1987). The cells involved in this antidepressant effect are still not identified. Desensitization of β-adrenoceptors by desipramine has been reported in vitro in primary astrocyte (Richardson and Hertz, 1983) and C6 rat glioma cell (Manji et al., 1991) cultures but these data are controversial (Ebisawa et al., 1988). The different culturing conditions used probably explain this discrepancy. For these reasons we have worked on neurons and astrocytes freshly isolated from rat brain. We examined the effects of chronic desipramine administration to the rats on β-adrenoceptor subtypes and on β-adrenoceptor coupling to G_s by determining the ratio of low- and high-affinity states.

2. Materials and methods

Acetylated trypsin (type V-S, from bovine pancreas), soybean trypsin inhibitor (type I-S, lyophilized), fructose, glucose, Ficoll 400, desipramine and isoproterenol were purchased from Sigma Chemical Co. (St. Louis, MO, USA). (−)-[^{3}H]CGP 12177 ((−)-4-(3-*t*-butylamino-2-hydroxypropoxy)-[5,7-^{3}H]benzimidazol-2-one; 51 Ci/mmol) was obtained from Amersham (Les Ulis, France). CGP 20712A (1-[2-((3-carbamoyl-4-hydroxy) phenoxy) ethylamino]-3-[4-(1-methyl-4-trifluoromethyl-2-imidazolyl) phenoxy]-2-propanol methane-sulfonate) was kindly provided by Ciba-Geigy (Basel, Switzerland) and ICI 118551 ((±)-1-[2,3-dihydro-7-methyl-1*H*-inden-4-yl) oxy]-3-[(1-methylethyl) amino]-2-butanol) by Imperial Chemical Industries (Macclesfield, UK).

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2.1. Desipramine treatment

Male Wistar rats, weighing 300–350 g at the end of drug administration, were housed at 22–24°C, four in each cage with a 12-h light/dark cycle. Food and water were freely available. Daily desipramine treatment (16 mg/kg) was given by intraperitoneal injection for 8 days. Saline solution was administered to control rats. Approximately 24 h after the last injection, the animals were killed by decapitation and brain tissue was rapidly removed. All animal use procedures were in strict accordance with the French Medicine Agency concerning animal experimentation (authorization No. 00748 delivered to J.P. Tillement).

2.2. Cell isolation

Cells were isolated from rat brains according to the method of Farooq and Norton (1978). After removal of the meninges, the forebrain and the cerebellum were sliced into 8–12 pieces with a McIlwain tissue chopper and incubated at 37°C with shaking in 2% Ficoll solution containing 0.1% trypsin for 70 min. Trypsinization was stopped by rinsing the slices with 0.1% soybean trypsin inhibitor. After the slices had been washed 4–5 times, disaggregation was achieved by aspiration through a nozzle in successive steps. The neuron- and astrocyte-enriched fractions were then obtained by differential centrifugation and finally by centrifugation through a Ficoll step-gradient. The cellular composition of these fractions was determined by immunocytochemistry.

2.3. Immunocytochemical analysis

Astrocytes were characterized with antibodies to glial fibrillary acidic protein (GFAP), a specific marker for astrocytes, neurons, with two different monoclonal antibodies (molecular weight 68 kDa and 160 kDa) to neurofilament proteins, microglia, with the antibody OX-42 which recognizes the antigen CD11b of macrophages and, finally, granulocytes and endothelial cells with the alkaline phosphatase test.

2.4. Binding experiments

Just before use, the cell fractions were suspended in Tris 50 mM, NaCl 120 mM, KCl 5 mM, $MgCl_2$ 3 mM pH = 7.4 at 37°C at a protein concentration of 0.5 mg/ml. Protein concentration was determined by the method of Lowry et al. (1951). Binding experiments were carried out with a total volume of 500 μl containing 400 μl of membrane suspension, the β-adrenoceptor labelling ligand (0.6–0.7 nM [^{3}H]CGP 12177), the competing drug as required and the incubation buffer. Non-specific binding was defined with 100 μM isoproterenol. Incubation time was 60 min at 37°C. The incubation was ended by adding 5 ml of cold buffer to each tube and rapidly filtering the contents under vacuum through Whatman GF/B filters. Each filter was washed with an additional volume of twice 5 ml of ice-cold buffer. The radioactivity of the filters was then measured in a liquid scintillation counter.

2.5. Data analysis

The binding parameters, K_i (inhibition constant) and B_{max} (maximal number of binding sites) were evaluated by means of non-linear regression using commercially available software (Micropharm, INSERM 1990; Morin et al., 1992).

3. Results

The neuronal fraction showed ~90% staining for the 68 and 160 kDa neurofilament proteins while 60–70% of the cells were GFAP-positive in the astrocyte fraction. Double labelling of the cells indicated very slight cross-contamination between astrocyte and neuronal fractions. The low cellular yield was due to the high purification of each fraction. Owing to the very low yield for cerebellar astrocytes, desipramine treatment effects could not be studied.

β-Adrenoceptors are expressed on both neurons and astrocytes but are more concentrated on astrocytes than on

Table 1
Desipramine effects on β-adrenoceptor densities of neurons and astrocytes isolated from rat brain

	B_{max} (fmol/mg protein)					
	β-Adrenoceptors		β_1-Adrenoceptors		β_2-Adrenoceptors	
	Control	Desipramine	Control	Desipramine	Control	Desipramine
Forebrain						
Astrocytes	27.36 ± 0.96	22.26 ± 0.58 [c]	23.82 ± 0.96	19.16 ± 0.59 [c]	3.55 ± 0.30	3.11 ± 0.20
Neurons	12.53 ± 0.48	10.69 ± 0.35 [b]	9.59 ± 0.51	7.88 ± 0.25 [b]	2.94 ± 0.36	2.77 ± 0.32
Cerebellum						
Neurons	4.86 ± 0.35	3.27 ± 0.51 [a]	1.28 ± 0.26	1.15 ± 0.18	3.58 ± 0.43	2.13 ± 0.35 [a]

Desipramine (16 mg/kg) was injected i.p. for 8 days. β-Adrenoceptors were labelled with [^{3}H]CGP 12177. [^{3}H]CGP 12177 binding was displaced by CGP 20712A, a selective antagonist of β_1-adrenoceptors. B_{max} (fmol/mg protein) are the means ± S.E.M. of 6–11 independent experiments and were compared by Student's t-test ([a] $P < 0.05$, [b] $P < 0.01$, [c] $P < 0.0005$).

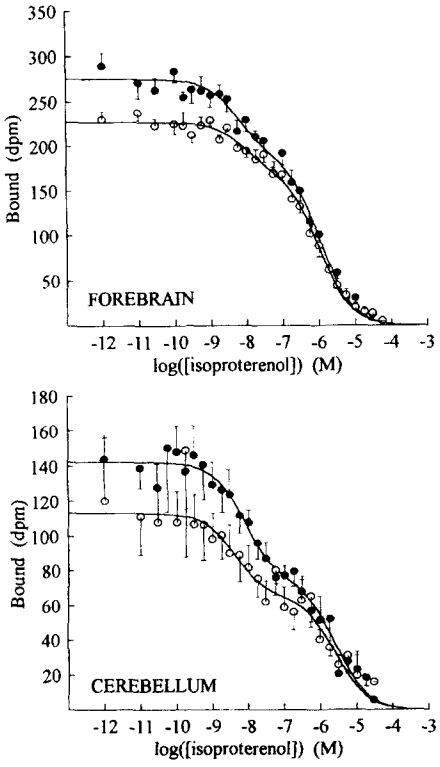

Fig. 1. Inhibition of [^{3}H]CGP 12177 binding by isoproterenol on neurons of desipramine-treated rats. [^{3}H]CGP 12177 (0.6 nM) was displaced by isoproterenol from neurons isolated from control (●) or desipramine-treated rat brains (○). Each competition curve is based on the mean ± S.E.M. of 6–11 independent experiments.

neurons and more in forebrain than in cerebellum (Table 1). Chronic desipramine administration induced down-regulation of β-adrenoceptors in both neurons and astrocytes (Table 1) corresponding to a 17% reduction of β-adrenoceptor number in the forebrain and 33% in the cerebellum. This inhibition affected the predominant β-adrenoceptor subtype, i.e. reduction of β_1-adrenoceptor density in forebrain and of β_2-adrenoceptors in cerebellum (Table 1).

β-Adrenoceptors were in the same low-affinity state in astrocytes ($K_i = 220$ nM) and in high- and low-affinity states in neurons ($K_{iH} = 4$ nM and $K_{iL} = 300$ nM). In cerebellar neurons, high- and low-affinity sites, which represent 47% and 53% of β-adrenoceptors respectively, were down-regulated after antidepressant treatment (Fig. 1) but the ratio of high/low-affinity sites remained the same. In forebrain, chronic desipramine administration only affected neuronal high-affinity β-adrenoceptors (Fig. 1). The ratio of high/low-affinity sites decreased from 33%/67% to 24%/76% after treatment. K_i remained constant in the two brain areas studied after desipramine administration.

4. Discussion

The presence of β-adrenoceptors in the central nervous system is now well established but their cellular localization is still under debate. In our previous study (Sapena et al., 1994), we had shown that β-adrenoceptors of the rat brain are much more concentrated on astrocytes than on neurons. The β-adrenoceptor subtypes, β_1 and β_2, coexist and are similarly distributed in these two cell types: β_2-adrenoceptors are predominant in the cerebellum (90%) and only represent 15% of the forebrain β-adrenoceptors (Minneman et al., 1981). Our present results showed that rat desipramine treatment causes a substantial reduction of brain β-adrenoceptors as previously described for homogenates (Argenti and D'Mello, 1994; McManus and Greenshaw, 1991). This down-regulation seems to be subtype-dependent. Stahl et al. (1987) and others already demonstrated that chronic desipramine administration to rats only reduced β_1-adrenoceptor density in the cerebral cortex. Our results are consistent with this since only the predominant β-adrenoceptor subtype in a tissue, namely β_1 in the forebrain, was affected by the desipramine treatment. We also observed down-regulation of the β_2-adrenoceptors in cerebellum neurons.

Two conformational states showing a low- and a high-affinity for agonists have been described for β-adrenoceptors (Levitzki, 1988). The high-affinity binding sites are sensitive to GTP. In a previous paper (Sapena et al., 1994), we have shown that β-adrenoceptors are all in the same conformational state of low affinity ($K_i = 220$ nM) in astrocytes but in high- ($K_{iH} = 4$ nM) and low- ($K_{iL} = 300$ nM) affinity states in neurons. The same results were found in the present study, suggesting that β-adrenoceptors are differently coupled to G-proteins in neurons and astrocytes.

In neurons, desipramine treatment reduces the number of both the high- and the low-affinity sites in the cerebellum and only the high-affinity site in the forebrain without affecting their corresponding affinity ($K_{iH} = 4$ nM and $K_{iL} = 300$ nM). The latter result is different from the results of Turkka et al. (1989) showing an increase in K_{iL}/K_{iH} ratio associated to β-adrenoceptor down-regulation in the hippocampus after chronic desipramine administration to rats. Similar results were observed by Manji et al. (1991): chronic exposure of C6 glioma cells to desipramine desensitizes β-adrenoceptors and increases the K_{iL}/K_{iH} ratio. We have no explanation for this discrepancy. In cerebellum, the β-adrenoceptors are mainly of the β_2 subtype and the two conformational states are affected by the desipramine treatment. In forebrain, where the β_1-adrenoceptor is the predominant subtype, only the high-affinity site density is reduced. All these results indicate that desipramine treatment decreases the β_1 high-affinity sites and both the β_2 high- and low-affinity sites. Desipramine thus seems to affect the two β-adrenoceptor subtypes of the neurons in different ways.

In conclusion, the present study demonstrated that desipramine treatment of rats down-regulates β-adrenoceptors to a similar extent in neurons and astrocytes and further emphasizes that the effects of desipramine are

subtype-dependent and differ between selected brain areas. Other studies will be required to elucidate the mechanisms accounting for the clinical effect of antidepressants. More particularly, it would be interesting to study the effects of such drugs on the effector systems coupled to the various β-adrenoceptors.

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