The significance of $\beta$-adrenoceptor down regulation in the desipramine action in the forced swimming test

Yoshimi Kitada, Tatsuo Miyauchi, Takashi Kosasa, and Susumu Satoh
Department of Pharmacology, Pharmaceutical Institute, Tohoku University, Aobayama, Sendai 980, Japan

Summary. The present studies were undertaken to clarify whether central $\beta$-adrenoceptor down regulation is responsible for the greater effect of chronic treatment with desipramine (DMI) compared with acute treatment in the forced swimming test in rats. Repetitive administration of DMI activated the rat behaviour pattern and consequently reduced the duration of immobility. The degree of activation depended on the length of treatment, i.e. no effect when given in a single dose, moderate effect when given subchronically (3 doses) and marked activation after chronic (31 doses) treatment. Chronic treatment with DMI also produced a decrease in $\textit{H}$-dihydroalprenolol ($\textit{H}$-DHA) binding site in the cerebral cortex. Acute stimulation of brain $\beta$-adrenoceptors by intracerebroventricular (i.c.v.) isoprenaline significantly, though partially, attenuated the behavioural effect of chronic DMI by $\beta_1$-adrenoceptor-related mechanisms. Similarly, chronic i.c.v. co-administration of atenolol or practolol, $\beta_1$-adrenoceptor antagonists, together with DMI attenuated both $\beta$-adrenoceptor down regulation and the behavioural activation by chronic DMI. On the other hand, chronic i.c.v. administration of isoprenaline, supposedly leading to down regulation of $\beta$-adrenoceptors, facilitated the activating behavioural effect of DMI, as a single dose became effective. Changes, however, in $\textit{H}$-DHA binding parameters in the cerebral cortex were not observed after chronic isoprenaline. These results suggest that down regulation of $\beta$-adrenoceptors in brain is responsible, at least in part, for the marked activatory effect of chronic DMI in the forced swimming test, possibly by reducing an inhibitory function of $\beta_1$-adrenoceptor mediated mechanisms.

Key words: Chronic desipramine — $\beta$-Adrenoceptor down regulation — Forced swimming test

Introduction

Long term treatment with antidepressants decreases the responsiveness to noradrenaline (NA) of NA receptor-coupled adenylyl cyclase (Vetulani and Sulser 1975; Vetulani et al. 1976; Mishra et al. 1980) and the density of $\beta$-adrenoceptors (Banerjee et al. 1977; Bergstrom and Kellar 1979a; Peroutka and Snyder 1980) in rat brain. The $\beta$-adrenoceptor down regulation is usually observed after chronic treatment with antidepressants, and thus has been believed to correspond with the therapeutic effect of the drugs, which take several days or weeks to develop (Oswald et al. 1972; Prange 1972). However, to our knowledge, there has been no report examining whether $\beta$-adrenoceptor down regulation is causally related to the behavioural effects of chronically administered antidepressants in depression model animals. Such data are essential for establishing the relevance of the neurochemical change with respect to the treatment of depression.

The forced swimming test in rats has been described by Porsolt et al. (1977, 1978) as a useful screening test for antidepressants. Non-pharmacological treatments that have proven efficacy in human depression (electroconvulsive shock, REM sleep deprivation or “enriched” environment), as well as tricyclic and atypical antidepressants, produce a positive effect in the test. We have previously reported that prolongation of active swim, and reduction of the duration of immobility as a result, which is potentiated after chronic treatment is an action specific to antidepressants (Kitada et al. 1981). Thus, analysis of the action of antidepressants in the forced swimming test may provide some insight into the mechanism of the clinical effects of the drugs. It has been reported that treatments which have shown to cause $\beta$-adrenoceptor down regulation such as chronic restraint stress (Stone and Platt 1982) and chronic electroconvulsive shock (Bergstrom and Kellar 1979b; Vetulani et al. 1976) elicit a positive response in the forced swimming test (Platt and Stone 1982; Porsolt et al. 1977). A causal relationship, however, between the $\beta$-adrenoceptor down regulation and the behavioural effect is not certain from these studies.

We have shown that the primary mechanism of the behavioural activatory action of subchronic and chronic DMI in the forced swimming test is the indirect activation of central $\alpha_1$-adrenergic mechanisms, and that the effect of subchronic DMI is tonically inhibited by $\beta_1$-adrenoceptor-mediated mechanisms (Miyauchi et al. 1981; Kitada et al. 1983a, b). Interestingly, it has been shown that $\beta$-adrenoceptors which are down regulated by chronic DMI are that of $\beta_1$- but not $\beta_2$-type (Minneman et al. 1979). This prompted us to speculate that the potentiation of the action of DMI after chronic treatment with the drug is caused by attenuated function of the inhibitory $\beta_1$-adrenoceptor-mediated mechanisms (Kitada et al. 1983a). The present studies were undertaken to test this hypothesis by examining (1) whether a manipulation which abolishes the DMI-induced $\beta$-adrenoceptor down regulation also inhibits the behavioural action of chronic DMI and (2) whether a manipulation, other than by antidepressants, which induces $\beta$-adrenoceptor down regulation facilitates the behavioural action of a single dose of DMI. It has been shown that chronic DMI-induced $\beta$-adrenoceptor down regulation in rat brain is inhibited by co-administration of propranolol,
a β-adrenoceptor antagonist (Wolfe et al. 1978; Asakura et al. 1982). On the other hand, chronic intracerebroventricular (i.c.v.) infusion of a β-agonist isoprenaline induces β-adrenoceptor down regulation like antidepressants in rat cerebral cortex (Wang and U’Prichard 1980).

Materials and methods

Animals and cannula implantation

Male Sprague Dawley rats (200—220 g), 7-week-old at the time of behavioural and biochemical measurements, were used for all experiments. They were housed in a room maintained at 21—23°C with a 12 h light-dark cycle and were allowed free access to food and water. For i.c.v. cannulation, animals were anesthetised with sodium pentobarbital (50 mg/kg, i.p.) and a stainless steel guide cannula with a inner obturator was stereotaxically implanted into the right lateral ventricle by the method of Kohno et al. (1981). After surgery, they were housed singly and at least 1 week was allowed for recovery from the surgical procedures before the drug treatments were begun.

Drug treatments

Experiment 1. Rats were injected i.p. with DMI (10 mg/kg) or saline twice daily (9:00 a.m. and 7:00 p.m.) for 15 days. On the 16th day, the final dose (31st) of DMI or saline was given to the rats between 9:00 and 11:00 a.m. and immobility was measured 5 h later. Isoprenaline (160 μg/rat, i.c.v.) was given in a single injection 15 min before the immobility measurement. Practolol (300 μg/rat, i.c.v.) was given 45 min before the injection of isoprenaline.

Experiment 2. Rats were treated chronically with DMI or saline and immobility was measured as described above. In these rats, atenolol (100 μg/rat, i.c.v.) or practolol (100 μg/rat, i.c.v.) was administered simultaneously with DMI for 15 days. On the 15th day, the last dose (30th) of the β-antagonists was given between 2:30 and 4:30 p.m. and immobility was measured 24 h later (5 h after the last dose of DMI). For comparison, DMI (20 mg/kg, i.p) or saline was given in 3 injections 24, 5 and 1 h before the immobility test.

Experiment 3. Rats were treated chronically with isoprenaline (60 μg/rat, i.c.v.) or saline (i.c.v.) twice daily (9:00 a.m. and 7:00 p.m.) for 15 days. On the 15th day, the last dose was given between 2:30 and 4:30 p.m. On the 16th day, 19 h after the last injection of isoprenaline, either DMI (20 mg/kg, i.p.) or saline was administered in a single injection and immobility was measured 5 h later.

Experiment 4. Rats were treated chronically with DMI, DMI + practolol, isoprenaline or saline for 15 days as described above. On the 16th day, 24 h after the last dose of the β-adrenergic drugs or 19—24 h after the last dose of DMI, the rats were killed by decapitation for 3H-DHA binding assay.

All drugs used in the present study were dissolved in 0.9% saline. Drugs were injected i.p. or i.c.v. in a constant volume of 5 ml/kg or 10 μl/animal, respectively. The doses are described in terms of the salt.

Measurement of immobility

The procedures were the same as described by Porsolt et al. (1978). Briefly, the rats were given two trials in which they were forced to swim in a cylinder from which they could not escape. There was a 24 h interval between the first and second trial. The first trial lasted 15 min and the second 5 min. The total duration of immobility was measured during the 5 min test of the second trial. In all instances, the immobility was measured between 2:00 and 6:00 p.m.

Binding experiments

Brains were removed and washed in ice-cold saline, and placed on an ice-cold glass plate. A transverse section was made at 3 mm caudal to the optic chiasma, and cerebral cortex was dissected from part of the brain rostral to the section. Immediately after dissection, the tissues were frozen and stored in liquid nitrogen. β-Adrenoceptor binding was quantified the method of Bylund and Snyder (1976). The cortex was homogenized in 20 volumes of 30 mmol/l Tris-HCl buffer containing 3 mmol/l MgCl₂, pH 8.0 at 25°C, using an Ultra Turrax homogenizer. After centrifugation (50,000 × g for 10 min at 4°C), the pellet was resuspended in 20 volumes of buffer and centrifuged again. The final pellet was resuspended in 100 volumes of buffer. Aliquots (0.88 ml) of tissue suspension (0.5—0.6 mg protein) were incubated, in triplicate, for 15 min at 25°C in the presence of 3H-DHA (0.25—4.0 nmol/l) alone or in combination with 20 μmol/l (+)-propranolol, in a final volume of 1.0 ml. The experiment was terminated by rapid filtration under vacuum through Whatman GF/B filters which were washed three times with 5 ml of cold buffer. The filters were dried for 1 h at 90°C. Radioactivity was counted by liquid scintillation spectrometry in 8 ml of toluene scintillation cocktail at an counting efficiency of about 53%. Specific binding was defined as the amount of isotope displaced by propranolol. The maximum number of binding sites (B_max) and the equilibrium dissociation constant (K_d) for 3H-DHA were determined by Scatchard analysis (Scatchard 1949). The protein content was measured by the method of Lowry et al. (1951).

Drugs and Chemicals used

Desipramine HCl (Pertofran, Nippon Ciba-Geigy, Takara-zuka, Japan), (+)-atenolol HCl and (+)-practolol HCl (ICI Pharma, Macclesfield, Great Britain), (+)-isoprenaline HCl (Nakarai Chemicals, Tokyo, Japan), (+)-[propyl-1,2,3-3H]-3H-dihydroalprenolol HCl (38.5 Ci/mmol, New England Nuclear, Boston, MA, USA) and (+)-propranolol HCl (Sigma Chemical, St. Louis, MO, USA).

Statistical analysis

The statistical analysis of the behavioural data were carried out by analysis of variance as described by Zivin and Bartko (1976). The biochemical data were analysed using two-tailed Student’s t-test.

Results

Repeted administration of DMI activated the rat behaviour and consequently reduced the duration of immobility. The