Korean J Physiol Pharmacol
Vol 1: 161–167, April, 1997

Neural Adaptation of Beta Adrenergic Receptor Subtypes after Chronic Imipramine Treatment: A Quantitative Autoradiographic Study

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This study compares the subtypes of central beta adrenergic receptors (ARs) of brains of untreated rats with those of imipramine-treated rats. Beta adrenergic receptors were measured by quantitative autoradiography of the binding of ^{3}H-dihydroalprenolol (^{3}H-DHA) in coronal sections of rat brain. Repeated treatment of rats with imipramine significantly reduced the binding of ^{3}H-DHA to beta-1 AR in many brain areas, especially throughout the cerebral cortex, hippocampus, thalamus, and amygdala. Significant reductions of the binding of ^{3}H-DHA to beta-2 AR were not found in any area of the brain. These data suggests that a selective down-regulation of beta-1 AR may be involved in the adaptive changes occurring after prolonged imipramine treatment.

Key Words: Beta adrenergic receptor, Rat brain, Autoradiography, Imipramine

INTRODUCTION

Imipramine is a potent inhibitor of the presynaptic uptake of norepinephrine and serotonin (Schildkaut & Kety, 1967; Langer et al, 1980 & Wood et al, 1886). The relationship between the acute inhibition of monoamine uptake and the therapeutic effects of imipramine are unclear, inasmuch as therapeutic benefits of imipramine are fully apparent only after chronic treatment. It suggests that the antidepressant effects of imipramine result from adaptive responses to chronic drug treatment and not from acute pharmacological actions of the drug (Mandell, 1975 & Stone, 1983). Clinical studies have shown that the course of antidepressant drug therapy (Katz et al, 1987). In addition, considerable differences in the time course of improvement of discrete symptoms of depressive illness occur during antidepressant drug administration indicates that some adaptive neural mechanism is responsible for the therapeutic effects of the drugs.

In animal studies, several neurotransmitter systems have been shown to exhibit adaptive responses to chronic antidepressant treatment. Beta adrenergic receptors (ARs) and serotonin-2 receptors have been shown to be down-regulated after chronic treatment with a wide range of antidepressant drugs (Baker & Greenshaw, 1989; Heninger & Charney, 1987). In addition recent data have shown that ligand binding to the N-methyl-D-aspartate (NMDA) receptor complex is altered after chronic treatment with antidepressant agents (Nowak et al, 1993). Whether such adaptive responses to repeated antidepressant treatment occur in human subjects — and, if they do occur, what role they play — is unknown. However, the well-documented adaptive responses of specific receptors provide valuable model systems for use in exploring the neurochemical consequences of antidepressant drug administration. Published autoradiographic studies of beta AR down-regulation by imipramine drug provide limited information (Biegon, 1986; Duncan et al, 1993 & Ordway et al, 1988). In order to explore more

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thoroughly the neuroanatomical selectivity in the rate of beta AR subtypes adaptation, we assessed the effects of chronic (14 and 21 days) imipramine treatment on regional ^{3}H-dihydroalprenolol (^{3}H-DHA) binding in the presence of beta-1 or beta-2 selective antagonist in subdivisions of selected brain regions using a high resolution autoradiographic technique.

METHODS

Animals

Male Wistar rats, five weeks old at the start of treatment, were used for all experiments. The rats had free access to food and water.

Drug administration

Imipramine HCl (15 mg/kg/day, i.p.) was administered once daily for 14 or 21 days to the experimental groups. The control rats received saline injection at the same times. Twenty four hours after the last injection, the rats were killed by decapitation and their brains were quickly removed. Brains were dipped into cold isopentane (−20°C) for 30 sec (to maintain gross morphology) and then quickly placed onto dry ice for 10 min. Brains were stored at −70°C until they were sectioned.

Rats used in the studies of saturation kinetics, inhibition analysis and normal anatomical distribution of beta AR received no treatment.

Tissue preparation

Brains were warmed to −20°C, and coronal sections (12 μm) were cut using a cryostat microtome (Reichert Histostat). Sections were taken at the level of plate 21, 30, and 59 according to the Stereotaxic Atlas of the Rat Brain (Paxinos & Watson, 1986). Sections were thaw-mounted onto gelatin-coated microscope slides, dried, and then stored at −70°C.

Quantitative autoradiography

Slide-mounted 12 μm tissue sections were removed from the −70°C freezer and were first preincubated in TM buffer (170 mM Tris-HCl, pH 7.4, 10 mM $MgCl_2$) for 10 min at room temperature. They were then incubated with ^{3}H-dihydroalprenolol (^{3}H-DHA , 4 nM) in the presence or absence of competing ligands for 40 min at room temperature in TM buffer. After incubation, sections were washed twice (10 min each time) in ice-cold TM buffer to remove as much of the nonspecifically associated ligand as possible without removing any specifically bound ligand. Sections were then quickly rinsed in ice-cold deionized water and dried by placing the slides on a 60°C slide warmer for 1 min. Dried sections were then placed in X-ray casettes and tightly apposed to Amersham Hyperfilm-^{3}H for 5 weeks to generate autoradiograms. The film was developed for 3 min in Kodak D-19 developer, washed for 30 sec in deionized water, fixed for 4 min in Kodak rapid fixer, rinsed in water for 10 min and air dried. Adjacent sections were stained with hematoxylin and eosin to identify brain regions. Films were analyzed and quantified using a computerized image analysis system which transforms the values of optical densities of brain image into values of receptor densities expressed in fmol/mg protein using Amersham autoradiographic ^{3}H-microscale as standard.

The binding of ^{3}H-DHA to beta-1 or beta-2 AR was defined as the binding of ^{3}H-DHA (4 nM) in the presence of the beta-2 antagonist, ICI-118,551 (50 nM) or the beta-1 antagonist, ICI-89,406 (70 nM), respectively, minus nonspecific binding. The nonspe-

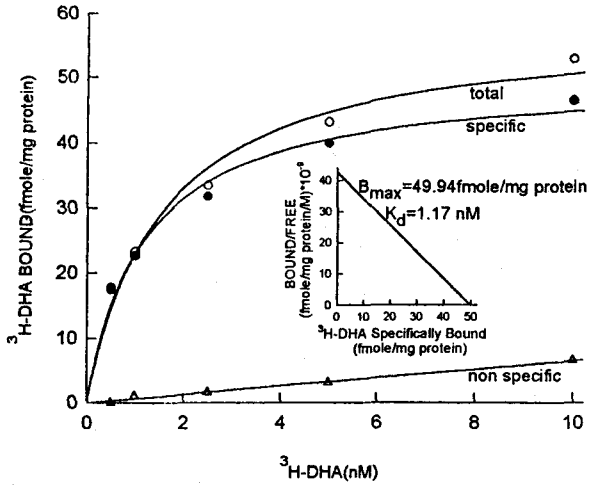

Fig. 1. Saturation binding of ^{3}H-dihydroalprenolol (^{3}H-DHA). 12 μm-thick sections were taken in accordance with plate 21 in Paxinos and Watson's stereotaxic atlas. Autoradiograms of caudate putamen were analyzed.

cific binding of ^{3}H-DHA was determined in the presence of dl-propranolol (1 μM).

RESULTS

Saturation studies

Saturation analysis (Fig. 1) was performed on serial slide-mounted tissue sections collected at the stereotaxic level of the caudate putamen (Paxinos & Watson, 1986; stereotaxic levels plate 21). Tissue sections collected at this level contained both beta-1 and beta-2 ARs. The serial nature of the sections ensured that a uniform amount of protein was present in each section.

It was found that ^{3}H-DHA bound with high affinity to a single population of binding sites over the concentration range studied (0.5～10.0 nM). The binding of ^{3}H-DHA to frozen sections of rat caudate was saturable, specific, and high affinity. Scatchard analysis of ^{3}H-DHA labeling revealed high affinity binding in the caudate (apparent K_d = 1.17±0.2 nM), which was saturated. The amount of ^{3}H-DHA binding in the presense of 1 μM dl-propranolol was consistently low, never exceeding 15% of total binding. An ^{3}H-DHA concentration of 4 nM was selected for further studies.

Table 1. Effects of chronic treatment of rats with imipramine (15mg/kg, daily) for 14 days or 21 days, or saline on the specific binding of ^{3}H-dihydroalprenolol (^{3}H-DHA) to beta-1 adrenergic receptor in rat brain

Region	^{3}H-DHA Binding(fmoles/mg protein)			Region	^{3}H-DHA Binding(fmoles/mg protein)		
	Saline	Imipramine 14 days	Imipramine 21 days		Saline	Imipramine 14 days	Imipramine 21 days
Somatosensory cortex				Zona Inserta	6.9±1.0	6.0±1.9	5.8±1.5*
layer 1-3	23.3±3.2	15.0±1.4*	15.3±2.3*	Hypothalamus			
layer 4	12.7±2.0	10.3±1.6*	10.4±1.7*	dorsal	11.7±1.3	9.0±1.3*	11.4±1.3
layer 5-6	10.7±1.4	8.4±1.4*	8.7±1.3*	ventral	10.3±1.4	7.9±1.5*	10.4±2.1
Cingulate cortex	19.0±3.2	12.2±2.0*	12.4±1.0*	lateral	9.9±0.9	7.2±1.0*	9.5±1.8
Caudate putamen	29.1±2.1	28.5±2.8	27.5±2.4	Amygdaloid			
Globus pallidus	13.3±1.7	11.6±2.6	12.1±2.4	basolateral	13.1±1.1	9.8±1.7*	8.7±1.6*
Subs. inominata	11.6±2.6	11.1±2.1	11.4±2.0	medial	11.7±1.3	8.8±1.6*	7.7±1.2*
Red nucleus	10.1±2.4	7.6±1.2*	8.5±1.3	Midbrain			
Med. preoptic area	11.8±2.2	8.9±1.5*	9.6±2.0	Med. geniculate	7.6±1.6	6.9±1.8	7.2±1.6
Lat. preoptic area	11.4±2.6	9.9±2.1	9.5±2.9	Sup. colliculus	16.8±2.1	15.9±2.8	15.8±3.1
Septal nuclei	6.9±1.9	5.4±1.1*	4.8±0.8*	Central gray	13.4±3.3	12.9±2.7	12.7±3.3
Hippocampus				Subs. nigra	20.0±3.9	21.2±3.6	19.5±2.9
CA1	9.5±1.9	7.1±1.4*	7.6±2.0*	Cerebellum			
CA2/CA3	7.4±1.2	5.5±1.8*	6.1±1.5*	molecular layer	3.6±1.0	3.2±1.3	3.8±1.1
dentate gyrus	12.6±1.8	10.6±1.2*	10.3±1.2*				
Thalamus							
ventrolateral	8.7±1.5	6.8±1.5*	7.5±1.5*				
centrolateral	11.2±2.6	8.0±1.4*	8.7±1.1*				
posterior	8.7±1.9	7.0±1.4*	7.3±1.5*				
central medial	9.4±2.2	7.3±2.0*	8.8±1.7*				

Results are means±SEM (n=12). 12 μm-thick sections were taken in accordance with the plate 21, 31, 41 and 59 in Paxinos and Watson's stereotaxic atlas. Binding to beta-1 AR was determined by incubation of brain sections with ^{3}H-DHA (4 nM) in the presence of the beta-2 selective antagonist ICI-118,551 (50nM). ^{3}H-DHA binding was determined by quantitative autoradiography for brain structures on each section. Five densitometric reading were taken for each structure on a single brain section.

* Value significantly different from corresponding value in saline-treated rats ($P<0.05$, Student t test)

Neuroanatomical selectivity in down regulation of beta-1 and beta-2 adrenergic receptors after chronic treatment with imipramine

To explore an extensive neuroanatomical analysis of the regulation of the subtypes of beta AR after imipramine treatment, receptor binding was evaluated by receptor autoradiography with ^{3}H-DHA. The binding of ^{3}H-DHA to beta-1 and beta-2 ARs in brain regions from animals treated with either imipramine or saline are shown in Table 1 and 2, respectively. Data were obtained from the quantitative analysis of selected areas of autoradiograms from rats treated with either imipramine or saline.

Treatment of rats with imipramine significantly reduced the specific binding of ^{3}H-DHA (4 nM) to beta-1 AR in many, but not all, regions of the brain. Of the 30 areas evaluated, 15 showed a significant imipramine-induced reduction in the binding of ^{3}H-DHA to beta-1 AR (Table 1). After 14 days of treatment with imipramine, significant reduction in the binding of ^{3}H-DHA to beta-1 AR was found in layers of the cerebral cortex, and in subregions of the hippocampus, thalamus, and amygdaloid. After 21 days of administration, significant reductions in the binding of ^{3}H-DHA to beta-1 AR were also found in the areas mentioned after 14 days of administration, with

Table 2. Effects of chronic treatment of rats with imipramine (15mg/kg, daily) for 14 days or 21 days, or saline on the specific binding of ^{3}H-dihydroalprenolol (^{3}H-DHA) to beta-2 adrenergic receptor in rat brain.

Region	^{3}H-DHA Binding(fmoles/mg protein)			Region	^{3}H-DHA Binding(fmoles/mg protein)		
	Saline	Imipramine 14 days	Imipramine 21 days		Saline	Imipramine 14 days	Imipramine 21 days
Somatosensory cortex				Zona Inserta	5.5±1.1	5.4±1.4	5.7±1.2
layer 1-3	5.5±1.1	5.0±0.8	5.3±0.9	Hypothalamus			
layer 4	8.6±1.7	7.9±0.9	8.1±1.4	dorsal	9.7±2.7	8.7±2.3	9.0±2.1
layer 5-6	6.8±1.2	6.4±0.9	6.3±1.4	ventral	8.3±2.0	7.2±2.0	8.0±1.8
Cingulate cortex	3.0±1.1	2.9±1.3	3.0±1.2	lateral	7.9±1.6	7.3±2.2	7.6±1.5
Caudate putamen	11.9±2.1	11.7±1.6	12.1±2.0	Amygdaloid			
Globus pallidus	9.8±2.8	9.9±2.6	8.4±2.3	basolateral	6.9±2.2	5.7±2.1	7.1±1.3
Subs. inominata	10.6±2.2	9.4±2.3	8.9±1.9	medial	7.4±2.3	6.9±2.1	7.4±2.5
Red nucleus	7.6±1.7	7.5±1.7	7.2±1.7	Midbrain			
Med. preoptic area	8.4±2.6	8.8±1.7	8.1±1.5	Med. geniculate	5.2±1.7	4.7±1.8	5.3±1.7
Lat. preoptic area	9.1±2.2	8.7±1.5	7.7±2.1	Sup. colliculus	13.1±3.8	11.4±2.8	12.2±2.4
Septal nuclei	5.2±1.4	5.4±1.2	4.4±1.3	Central gray	9.6±2.8	9.5±2.9	10.6±2.8
Hippocampus				Subs. nigra	17.1±4.1	16.0±4.1	16.2±4.2
CA1	4.1±1.7	4.0±1.5	4.2±1.6	Cerebellum			
CA2/CA3	5.4±1.5	4.8±1.2	5.0±1.6	molecular layer	23.1±2.2	22.1±2.5	22.3±2.6
dentate gyrus	6.6±1.6	7.2±1.2	6.6±0.9	granular layer	6.7±1.6	6.6±0.9	6.6±1.5
Thalamus							
ventrolateral	5.0±1.3	4.7±1.0	5.2±1.4				
centrolateral	10.1±2.2	9.3±1.9	9.9±2.3				
posterior	5.8±1.2	5.4±1.0	5.8±1.2				
central medial	5.7±1.0	4.6±1.4	6.2±1.0				

Results are means±SEM (n=12). 12 μm-thick sections were taken in accordance with the plate 21, 31, 41 and 59 in Paxinos and Watson's stereotaxic atlas. Binding to beta-2 AR was determined by incubation of brain sections with ^{3}H-DHA (4 nM) in the presence of the beta-1 selective antagonist ICI-89,406 (70nM). ^{3}H-DHA binding was determined by quantitative autoradiography for brain structures on each section. Five densitometric reading were taken for each structure on a single brain section.

No significant differences at $P<0.05$ were found between imipramine- and saline-treated rats.

the exception of the subregions of hypothalamus. No changes in the binding of ^{3}H-DHA to beta-1 AR were observed in the caudate putamen, globus pallidus, cerebellum, and several other areas of the brain (Table 1). Significant reductions of the binding of ^{3}H-DHA to beta-2 AR were not found in any area of the brain (Table 2). Thus, the binding of ^{3}H-DHA to beta-1 AR were decreased significantly in several areas of the brain, with different rate of adaptive response, whereas no significant changes were found in the amount of ^{3}H-DHA bound to beta-2 AR.

DISCUSSION

Down-regulation of the beta ARs was one of the first recognized apative responses induced by antidepressant drugs (Vetulani et al, 1976). In rats, this phenomenon is a common effect produced by a wide range of antidepressants with disparate pharmacological properties (Heninger & Charney, 1987; Baker & Greenshaw, 1989). Although the involvement of beta AR down-regulation in clinical antidepressant action is uncertain, this measure provides a model of neural adaptation produced by chronic antidepressant drug treatment.

The present autoradiographic study demonstrates that the selective down-regulation of brain beta-1 AR after chronic imipramine treatment is neuroanatomically selective even within subdivisions of major brain regions. In previous studies from other laboratories, 10-14 days of imipramine treatment showed no effect in the hypothalamus and 21 days of imipramine treatment produced a reduction in beta AR binding in several hypothalamic regions. The different findings between those studies and the present investigation probably relate to differences in specific hypothalamic regions measured and species of rats. The mechanism responsible for neuroanatomical differences in the rate of imipramine-induced beta-1 AR down-regulation is unknown. The observed neuroanatomical specificity is not explained by the topographic distribution of binding sites for imipramine and desipramine, as measured *in vitro* (Duncan et al, 1991, 1992) or by the regional distribution of the drugs *in vivo* (Duncan et al, 1991). And there is no apparent relationship between the extent of noradrenergic innervation and the treatment period required for beta-1 AR adaptation. (Swanson & Hartman, 1975). It is possible that the adaptation of beta ARs after chronic imipramine treatment is related more closely to the regional differences in release and turnover rates of norepinephrine rather than the extent of noradrenergic innervation. Unfortunately, there are presently inadeguate data available on regional rates of norepinephrine turnover to judge this possibility. Further work is required to understand the mechanisms responsible for neuroanatomical variations in beta-1 AR adaptation after antidepressant drug treatment.

The down-regulation of beta-1 AR by diverse antidepressant drugs should not be interpreted as a down-regulation of noradrenergic function. In fact, abundant electrophysiological and behavioral evidence indicates that there can be enhanced sensitivity to norepinephrine after chronic antidepressant treatment (Menkes & Aghajanian, 1981; Plaznik & Kostowski, 1984). The physiological role of norepinephrine appears to be to modulate responses to both excitatory and inhibitory transmitters, such as glutamate and gamma-aminobutyric acid (Woodward et al, 1979). Such physiological actions of norepinephrine are mediated by multiple receptors, including beta-1, beta-2, alpha-1 and alpha-2 AR subtypes, while alpha-ARs and beta-ARs frequently exhibit functional antagonism (Szabadi, 1979). The net consequences of altered sensitivity of beta-1 AR for neuromodulatory effects of norepinephrine *in vivo*, as related to electrophysiological responsiveness, are largely unexplored. In future studies, it will be of interest to examine behavioral effects in the forced swim test after injection of imipramine into specific brain regions that respond to chronic imipramine treatment with a down-regulation of beta ARs. Congruence between regional receptor adaptation and brain sites that support positive behavioral responses in animal screens for antidepressant drug action may provide evidence for involvement of specific brain regions in therapeutic actions of antidepressant drugs.

The rate of beta adrenergic receptor adaptation in response to antidepressant drug treatment can be accelerated by various pharmacological and nonpharmacological treatments. Coadministration of imipramine and electroconvulsive shock produced greater reductions in frontal cortical beta adrenergic receptor binding than did either independent treatment (Paul et al, 1991). Other preclinical investigations have found that coadministration of imipramine and alpha-2 adrenergic antagonists (Scott & Crews, 1983; Kendall

et al, 1982) or corticotropin(Kendall et al, 1982) can accelerate the down-regulation of beta adrenergic receptors. These findings raise the interesting possibility that combinations of antidepressnat medication with other pharmacological agents, electroconvulsive shock could be therapeutically useful. More clinical studies are needed in different patient populations to provide definitive assessment of the potential the apeutic utility of combining antidepressant drugs with other pharmacological and nonpharmacological treatment for the treatment of depression. Preclinical investigation of the rate and degree of adaptive responses produced by different combinations of antidepresant treatments could provide a rational basis to explore novel treatment regimens.

ACKNOWLEDGEMENT

This work was supported by an Interdisciplinary Research Grant from the Korea Science and Engineering Foundation (KOSEF).

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