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Effect of alterations in extracellular norepinephrine on adrenoceptors: a microdialysis study in freely moving rats

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Abstract

Chronic electroshock treatment (once daily for 12 days) increases extracellular norepinephrine in the frontal cortex and hippocampus as measured by microdialysis. This chronic treatment produced an elevation of basal norepinephrine overflow into extracellular space while both the first and the twelfth treatments produced a transient increase in norepinephrine overflow of about 40 min. Acutely, desmethylimipramine (10 mg/kg) treatment significantly increased extracellular norepinephrine. While chronic desmethylimipramine (once daily for 10 days) increased basal overflow of norepinephrine in the frontal cortex and hippocampus, the tenth daily administration of desmethylimipramine did not produce a statistically significant increase in extracellular norepinephrine. Both daily electroshock and daily desmethylimipramine produced down regulation of β-adrenoceptors in the hippocampus and the frontal cortex. Chronic electroshock caused up regulation of α-adrenoceptors in the frontal cortex but not in the hippocampus while chronic desmethylimipramine administration did not alter α-adrenoceptors in either structure. Depletion of norepinephrine with reserpine or with 6-hydroxydopamine prevented the down regulation of β-adrenoceptors while depletion of this neurotransmitter did not prevent the electroshock-induced up regulation of α-adrenoceptors in the frontal cortex. These data suggest that down regulation of β-adrenoceptors is mediated through increases in extracellular norepinephrine. In contrast, up regulation of α-adrenoceptors appears to be independent of norepinephrine release and does not require the presence of noradrenergic neurons in order to be induced by electroshock.

Keywords: Norepinephrine; Microdialysis; β-Adrenoceptor; α-Adrenoceptor; Hippocampus; Frontal cortex; Electroshock; Desmethylimipramine; (Rat)

1. Introduction

Stimulation of noradrenergic neurons electrically or through physiologic means increases the release and utilization of norepinephrine. A seizure is a transient, synchronous and rhythmic firing of populations of central nervous system neurons (McNamara, 1994). In mammals, seizures can occur naturally or be induced by a number of stimuli including electrical stimulation (Glue et al., 1990) and a variety of drugs (Loscher and Schmidt, 1988). Seizures produce a number of short and long term biochemical changes in brain. In the short term, seizures cause release of many neurotransmitters. In the longer term, seizures result in induction of selected enzymes and in the up or down regulation of receptors.

Electroconvulsive shock can cause seizures that are accompanied by stimulation of noradrenergic neurons resulting in increases in norepinephrine synthesis and turnover (Kety et al., 1967; Gleiter and Nutt, 1989). Similarly, electroconvulsive shock increases extracellular norepinephrine as measured by microdialysis (Jobe et al., 1995). Central adrenoceptor systems also are affected by repeated electroconvulsive seizures. A consistent finding in many brain areas is down regulation of β-adrenoceptors and up regulation of α-adrenoceptors following repeated

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electroconvulsive seizures (Bergstrom and Kellar, 1979; Pandey et al., 1979; Kellar and Bergstrom, 1983; Vetulani et al., 1983; Stockmeier et al., 1987; Gleiter and Nutt, 1989; Glue et al., 1990).

Desmethylimipramine increases extracellular and synaptic norepinephrine by blocking the reuptake of the neurotransmitter into noradrenergic neurons. This was demonstrated earlier using exogenously administered radio labeled norepinephrine (Glowinski and Axelrod, 1964) and has been confirmed more recently with microdialysis (Yan et al., 1993a). As was the case with chronic electroconvulsive shock, chronic treatment with desmethylimipramine causes down regulation of β-adrenoceptors (Saraiya et al., 1978; Riva and Creese, 1989a). However, unlike electroshock chronic desmethylimipramine treatment does not produce up regulation of α-adrenoceptors (Stockmeier et al., 1987).

In order to investigate further the role of synaptic norepinephrine in up and down regulation of receptors, we also employed two drugs known to deplete brain norepinephrine, reserpine and 6-hydroxydopamine. Reserpine irreversibly inactivates synaptic vesicles resulting in norepinephrine depletion and 6-hydroxydopamine is a neurotoxin with the capacity to destroy noradrenergic neurons (U'Prichard et al., 1979; Stockmeier et al., 1987; Giralt and Garcia-Sevilla, 1989; Kostrzewa, 1989).

The purpose of these experiments was to clarify further the role of extracellular norepinephrine in the up and down regulation of α- and β-adrenoceptors.

2. Materials and methods

2.1. Materials

Norepinephrine, Na_2EDTA, sodium phosphate, octanesulfonic acid, Trizma base, ascorbic acid, EGTA, NaCl, $MgCl_2$, polyethylenimine, DL-propranolol and phentolamine were obtained from Sigma (St. Louis, MO). Acetonitrile was obtained from Merck (Darmstadt, Germany). (−)-[^{3}H]dihydroalprenolol (84.0 Ci/mmol) and [7-methoxy-^{3}H]prazosin (78.0 Ci/mmol) were obtained from Amersham (Aylesbury, UK). All chemicals were reagent grade. Deionized water was used in the preparation of reagents.

2.2. Animals

Male Sprague–Dawley rats weighing 230–270 g, were used in this study. Animals were housed at 21 ± 3°C, 40–60% relative humidity and were maintained under 12 h light/12 h dark conditions with ad libitum access to food and water.

2.3. Experimental design

Eight groups of animals were prepared: (1) sham and electroshock groups; (2) saline and desmethylimipramine (10 mg/kg, i.p. daily for 10 days) treated groups; (3) reserpine-pretreated (5 mg/kg in methylcellulose suspension i.p. every third day for 12 days) sham and electroshock (were started after the first reserpine injection) groups; (4) 6-hydroxydopamine-pretreated (250 μg/20 μl, i.c.v. daily for 2 days) sham and electroshock groups. Tonic extensor convulsions were induced daily for 12 consecutive days via an electrical stimulus (electroshock) of 70 mA (60 Hz) administered for 0.5 s through ear-clip electrodes (Swinyard, 1972). This stimulus was produced by a constant current electroshock seizure apparatus (ME-5300, Metro Scientific, Farmingdale, NY). The sham groups were subjected to the same treatment as the electroshock groups except that no current was passed. Half of the animals were sacrificed by decapitation on the day after completion of each treatment protocol, and the brains were immediately removed into ice-chilled plates. The frontal cortex and hippocampus were dissected and stored at −70°C until the day of the densities of α- and β-adrenoceptors were measured by receptor binding assay. The other half of the animals was used in the microdialysis study. Animals were started on microdialysis so that the electroshock and desmethylimipramine treatments administered to the chronic animals were initiated at approximately the same time of day on each of the days of the experiment.

2.4. α-Adrenoceptor binding assay

All brain tissues were homogenized in buffer solution (50 mM Trizma base, 5 mM EGTA, 10 mM $MgCl_2$, pH 7.6; 50× tissue volume), using the Tekmar Tissumizer (Cincinnati, OH) set at seven for 20 s. The homogenates were centrifuged at 40,000 × *g* for 10 min at 4°C and then the process was repeated two times. The final tissue pellets were re-suspended in fresh buffer (w/v, 1:200 for frontal cortex, 1:125 for hippocampus). α-Adrenoceptor binding sites were measured in aliquots of tissue equivalent to 5 mg frontal cortex and 8 mg hippocampus. Tissues were incubated in duplicate for 30 min at 25°C with [7-methoxy-^{3}H]prazosin (0.03–1.20 nM). Total incubation volume was 2.0 ml and 1.0 μM phentolamine was used for non-specific binding. [^{3}H]prazosin bound to the membrane was separated by rapid filtration through a 0.3% polyethylenimine presoaked Whatman GF/B filter and washed three times with 5 ml ice-cold 10 mM Tris buffer, using a modified Brandel cell harvester (Gaithersburg, MD). Each filter was placed in a counting vial to which 10 ml of scintillation cocktail was added and counted in a liquid scintillation counter. The binding data were analyzed by

the iterative curve-fitting program LIGAND (Munson and Rodbard, 1980).

2.5. β-Adrenoceptor binding assay

All brain tissues were homogenized in buffer solution (50 mM Trizma base, 5 mM EDTA, 100 mM NaCl, pH 8.0) and centrifuged as described above for α-adrenoceptors. The final tissue pellets were re-suspended in fresh buffer (w/v, 1:50 for frontal cortex, 1:62.5 for hippocampus). β-Adrenoceptor binding sites were measured in aliquots of tissue equivalent to 10 mg frontal cortex and 8 mg hippocampus. Tissues were incubated in triplicate for 30 min at 25°C with [^{3}H]dihydroalprenolol (0.06–6.20 nM). Total incubation volume was 0.52 ml and the non-specific binding was floated and calculated by LIGAND program (Riva and Creese, 1989b). [^{3}H]dihydroalprenolol bound to the membrane was separated by rapid filtration through a 0.3% polyethylenimine presoaked Whatman GF/B filter and washed three times with 5 ml ice-cold 50 mM Tris buffer, using a modified Brandel cell harvester (Gaithersburg, MD). Each filter was placed in a counting vial to which 10 ml of scintillation cocktail was added and counted in a liquid scintillation counter. The binding data were analyzed by the iterative curve-fitting program LIGAND (Munson and Rodbard, 1980).

2.6. Microdialysis

Rats were prepared for microdialysis studies as previously described (Yan et al., 1993a). Briefly, loop type microdialysis probes were constructed from cellulose acetate dialysis fibers (I.D. 215 ± 15 μm, molecular weight cutoff = 6000; Spectrum Medical Industries, Los Angeles, CA) attached to fused silica tubing (Polymicro Technologies). The dialysis area was 4 or 6 mm long with the dialysis probes folded in half so that the dialyzed brain area was 2 or 3 mm deep.

To place guide cannulae, animals were anesthetized with ketamine (500 mg/kg, i.m.) and xylazine (100 mg/kg, i.m.). While anesthetized, the rats were placed in a Kopf stereotaxic frame and a 22-gauge guide cannula was affixed to the animal's skull with dental acrylic anchored by machine screws. The guides were placed over the frontal cortex and hippocampus without penetrating the dura. The coordinates relative to bregma for hippocampus were anteroposterior −4.2, L 2.0 mm while for frontal cortex they were anteroposterior +3.2, L 0.8 mm relative to bregma (Paxinos and Watson, 1986). Animals were allowed to recover for 5 days prior to the actual microdialysis experiments.

On the experimental day, the microdialysis probe was inserted into the guide and directed to the frontal cortex and hippocampus with the tip 5.2 and 4.2 mm below the dura, respectively. Rats were then placed into Plexiglas chambers and allowed to move freely about. Probes were perfused at a constant flow rate of 1.0 μl/min with artificial cerebrospinal fluid which contained (in mM) Na^+ (150), K^+ (3.0), Ca^{2+} (1.2), Mg^{2+} (0.8), Cl^- (155). Dialysis measures shown were taken after a stable baseline had been obtained.

For norepinephrine analysis, dialysates were directly injected into a high performance liquid chromatography (HPLC) with electrochemical detection as was described previously (Yan et al., 1993b). The HPLC system was an ESA (Bedford, MA) solvent delivery system (Model 580) consisting of a dual piston pump, an ESA MD-150 column (3 μm, ODS, 150 × 3.2 mm) and a Coulochem II electrochemical detector (Model 5200A). Detector output was recorded on a strip chart recorder. The mobile phase consisted of 75 mM NaH_2PO_4, 1.7 mM sodium octanesulfonic acid, 20 μM EDTA, 100 μl/l triethylamine (pH 3.1 with H_3PO_4) and 9% acetonitrile and was delivered through the system at 1 ml/min.

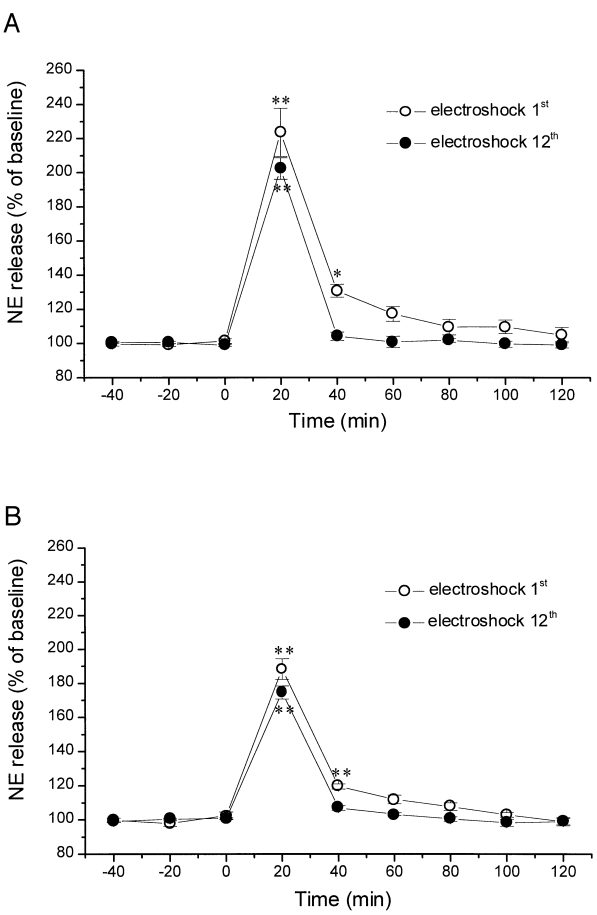

Fig. 1. (A and B) Norepinephrine release after first (open circle) and twelfth (closed circle) electroshock treatment, expressed as percent (%) of basal release. For data in panel A the microdialysis probe was in the frontal cortex. For panel B the microdialysis probe was in the hippocampus. Each symbol represents the mean ± S.E.M. of five rats. Electroconvulsive shock was given at time 0 min. * Indicates a significant difference from the baseline (* $P < 0.05$; ** $P < 0.01$).

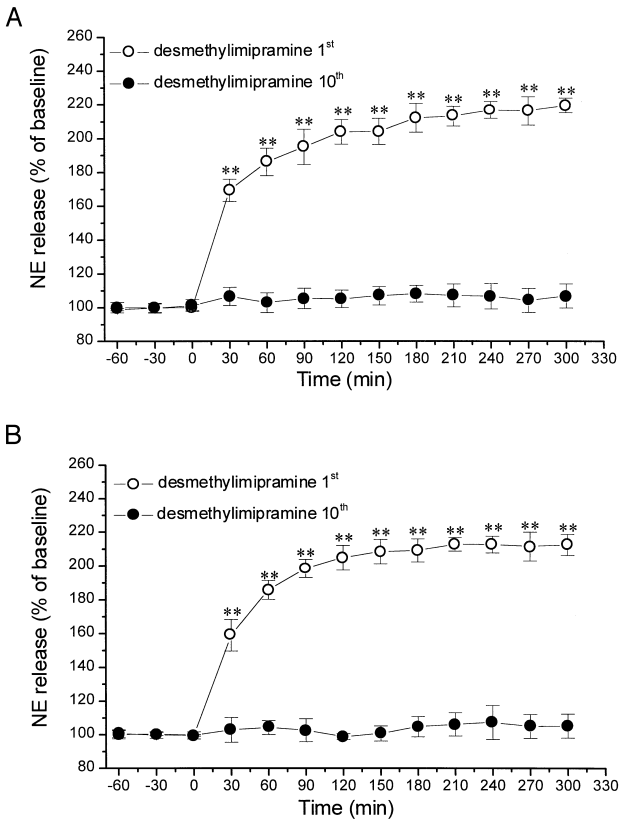

Fig. 2. (A and B) Norepinephrine release after first (open circle) and tenth (closed circle) desmethylimipramine treatment, expressed as percent (%) of basal release. For data in panel A the microdialysis probe was in the frontal cortex. For panel B the microdialysis probe was in the hippocampus. Each symbol represents the mean ± S.E.M. of five rats. Desmethylimipramine injection was given at time 0 min. * Indicates a significant difference from the baseline ($* P < 0.05$; $** P < 0.01$).

2.7. Statistical analysis

Changes in extracellular norepinephrine levels after the treatment were expressed as percentage of the mean basal output obtained in each individual rat. All data were analyzed by one-way analysis of variance (ANOVA) followed by Newman–Keuls' test or unpaired Student's *t*-test. Each value was expressed as the mean ± S.E.M. and statistical significance was accepted for $P < 0.01$ or $P < 0.05$.

3. Results

3.1. Microdialysis

Fig. 1 shows the effect of electroshock on extracellular norepinephrine in frontal cortex (panel A) and hippocampus (panel B). Data are expressed as percent of baseline and are shown for animals as they received a single electroshock treatment and for animals as they received the twelfth daily electroshock treatment. In comparison with baseline, both the single electroshock and the twelfth electroshock produced a transient and statistically significant increase in extracellular norepinephrine that lasted approximately 40 min.

Fig. 2 shows the effect of desmethylimipramine treatment on extracellular norepinephrine in frontal cortex (panel A) and hippocampus (panel B). Data are expressed as percent of baseline and are shown for animals as they received a single desmethylimipramine treatment and for animals as they received the tenth daily desmethylimipramine treatment. In comparison with baseline, the single desmethylimipramine treatment caused statistically significant elevation in extracellular norepinephrine that was sustained for at least 5 h. In contrast, the tenth daily desmethylimipramine treatment did not produce a statistically significant increase in extracellular norepinephrine.

Table 1 shows basal norepinephrine in each of the eight treatment groups prior to the last treatment in the series on the day of the last treatment. Chronic desmethylimipramine treatment and chronic electroshock treatment both produced statistically significant increases in basal norepinephrine overflow as measured by microdialysis. Thus, chronic electroshock increased basal norepinephrine release (Table 1) and both the first and twelfth electroshock

Table 1
Extracellular norepinephrine as measured by microdialysis[a]

Treatment regimen	NE basal release (fmol/μl)	
	Frontal cortex	Hippocampus
Sham	0.933 ± 0.033 (5)	0.966 ± 0.019 (5)
Electroshock	1.652 ± 0.101 (5)[b]	1.459 ± 0.042 (5)[b]
Saline	0.899 ± 0.026 (5)	0.921 ± 0.025 (5)
Desmethylimipramine	1.745 ± 0.092 (5)[b]	1.562 ± 0.053 (5)[b]
Reserpine + sham	below detection limit	below detection limit
Reserpine + electroshock	below detection limit	below detection limit
6-Hydroxydopamine + sham	below detection limit	below detection limit
6-Hydroxydopamine + electroshock	below detection limit	below detection limit

[a] Each numerical values in a single cell of the table represents the mean ± S.E.M. (*N*).
[b] Indicates a significant difference ($P < 0.01$) from the sham or saline group within the same set.

Table 2
β-Adrenoceptors [^{3}H]dihydroalprenolol binding[a]

Treatment regimen	Frontal cortex		Hippocampus	
	B_{max} (pmol/g tissue)	K_d (nM)	B_{max} (pmol/g tissue)	K_d (nM)
Sham	8.12 ± 0.12 (5)	0.57 ± 0.05 (5)	3.17 ± 0.14 (6)	0.63 ± 0.03 (6)
Electroshock	5.69 ± 0.08 (6)[b]	0.48 ± 0.02 (6)	2.43 ± 0.06 (6)[b]	0.52 ± 0.03 (6)
Saline	7.67 ± 0.15 (5)	0.68 ± 0.03 (5)	2.78 ± 0.09 (5)	0.66 ± 0.03 (5)
Desmethylimipramine	5.27 ± 0.26 (5)[b]	0.61 ± 0.04 (5)	1.78 ± 0.02 (4)[b]	0.62 ± 0.04 (4)
Reserpine + sham	9.77 ± 0.56 (5)[c]	0.57 ± 0.05 (5)	3.12 ± 0.26 (5)	0.48 ± 0.06 (5)
Reserpine + electroshock	8.59 ± 0.21 (5)	0.61 ± 0.06 (5)	3.11 ± 0.32 (5)	0.49 ± 0.08 (5)
6-Hydroxydopamine + sham	10.43 ± 0.17 (6)[c]	0.56 ± 0.01 (6)	3.98 ± 0.13 (5)[c]	0.44 ± 0.01 (5)
6-Hydroxydopamine + electroshock	9.93 ± 0.19 (5)	0.57 ± 0.01 (5)	4.08 ± 0.18 (5)	0.46 ± 0.02 (5)

[a] Each numerical values in a single cell of the table represents the mean ± S.E.M. (N).
[b] Indicates a significant difference ($P < 0.01$) from the sham or saline group within the same set.
[c] Indicates a significant difference ($P < 0.01$) from the non-treated sham and saline groups.

produced further increases in norepinephrine release above baseline (Fig. 1). Desmethylimipramine caused an increase in basal norepinephrine release that was evident with the tenth desmethylimipramine dose (Table 1). In contrast to electroshock, desmethylimipramine produced an increase above baseline with the first dose but there was no further increase above baseline with the tenth dose (Fig. 2).

In the reserpine pretreated and the 6-hydroxydopamine pretreated animals, basal norepinephrine levels were below detection limits. Similarly, both reserpine pretreatment and 6-hydroxydopamine pretreatment decreased the electroshock-induced levels of extracellular norepinephrine to concentrations that were below the detection limits for this assay procedure.

3.2. β-Adrenoceptor density

The β-adrenoceptor densities (B_{max}) and apparent dissociation constants (K_d) are summarized in Table 2. Chronic electroshock significantly decreased the B_{max} for dihydroalprenolol binding in the frontal cortex and hippocampus ($P < 0.01$). Chronic desmethylimipramine treatment also significantly decreased the B_{max} for dihydroalprenolol binding in the frontal cortex and hippocampus when compared with saline treatment. Both reserpine and 6-hydroxydopamine treatment increased B_{max} for dihydroalprenolol binding in the frontal cortex but not in the hippocampus. In the reserpine or 6-hydroxydopamine pretreated animals chronic electroshock treatment failed to decrease the B_{max} for dihydroalprenolol binding either in the frontal cortex or in the hippocampus. No significant difference in K_d values was detected in either region of the brain in any of the groups.

3.3. α-Adrenoceptor density

The α-adrenoceptor densities (B_{max}) and apparent dissociation constants (K_d) are summarized in Table 3. Chronic electroshock treatment significantly increased the B_{max} for prazosin binding in the frontal cortex ($P < 0.01$), but in the hippocampus the B_{max} was not changed. Chronic desmethylimipramine treatment failed to alter the B_{max} for prazosin binding compared to saline treatment. In reserpine or 6-hydroxydopamine pretreated animals, chronic electroshock significantly ($P < 0.01$) increased the B_{max} for

Table 3
α-Adrenoceptors [^{3}H]prazosin binding[a]

Treatment regimen	Frontal cortex		Hippocampus	
	B_{max} (pmol/g tissue)	K_d (nM)	B_{max} (pmol/g tissue)	K_d (nM)
Sham	13.48 ± 0.28 (5)	0.0764 ± 0.0048 (5)	4.75 ± 0.12 (5)	0.0870 ± 0.0047 (5)
Electroshock	19.23 ± 0.50 (5)[b]	0.0812 ± 0.0041 (5)	4.83 ± 0.14 (5)	0.0818 ± 0.0046 (5)
Saline	13.06 ± 0.30 (6)	0.0968 ± 0.0056 (6)	4.93 ± 0.06 (5)	0.1332 ± 0.0067 (5)
Desmethylimipramine	12.90 ± 0.10 (6)	0.1058 ± 0.0067 (6)	5.03 ± 0.14 (5)	0.1368 ± 0.0075 (5)
Reserpine + sham	21.60 ± 0.20 (5)	0.0876 ± 0.0014 (5)	7.71 ± 0.21 (5)	0.1250 ± 0.0063 (5)
Reserpine + electroshock	27.96 ± 1.02 (5)[b]	0.0830 ± 0.0019 (5)	7.92 ± 0.35 (5)	0.1296 ± 0.0052 (5)
6-Hydroxydopamine + sham	21.20 ± 0.50 (5)	0.0902 ± 0.0040 (5)	7.22 ± 0.13 (5)	0.1176 ± 0.0043 (5)
6-Hydroxydopamine + electroshock	31.42 ± 1.21 (5)[b]	0.0802 ± 0.0035 (5)	7.30 ± 0.15 (5)	0.1182 ± 0.0046 (5)

[a] Each numerical values in a single cell of the table represents the mean ± S.E.M. (N).
[b] Indicates a significant difference ($P < 0.01$) from the sham or saline group within the same set.

prazosin binding in the frontal cortex ($P < 0.01$), but in the hippocampus the B_{max} was not significantly changed. No significant difference in K_d values was detected in either region of the brain in any of the groups.

4. Discussion

Our present work confirms that both electroshock and desmethylimipramine treatment acutely increase extracellular norepinephrine in the frontal cortex and the hippocampus (Figs. 1 and 2). Other authors have shown that, in anesthetized animals, acute electroshock produces marked elevations in the overflow of norepinephrine into extracellular fluid of the frontal cortex and the hippocampus (Glue et al., 1990; Thomas et al., 1992). Also, we have previously shown that acute electroshock produces very rapid increases in extracellular norepinephrine in the superior colliculus of unanesthetized, freely moving rats (Jobe et al., 1995).

Our current work shows that on chronic administration, electroshock treatment produced an elevation of basal overflow of norepinephrine into extracellular space with both the first and twelfth treatments producing a further, transient increase above baseline in norepinephrine overflow of about 40-min duration (Fig. 1). A different response pattern was seen with chronic administration of desmethylimipramine. While chronic desmethylimipramine produced an increase in basal overflow of norepinephrine in the frontal cortex and hippocampus, the tenth daily administration of desmethylimipramine did not produce a further increase above baseline in extracellular norepinephrine (Fig. 2). Thus, the responses to chronic electroshock and chronic desmethylimipramine treatment differ. Chronic electroshock increased both basal and stimulated release while chronic desmethylimipramine increased basal release without a further increase in norepinephrine release on the tenth dose. The finding that the tenth daily dose of desmethylimipramine did not cause an elevation of norepinephrine above baseline confirms the results of Tanda et al. (1996). These investigators found that on chronic administration, desmethylimipramine increased basal overflow of norepinephrine. As in the current study, they also found that a challenge dose of desmethylimipramine administered on day 14 did not cause a further increase in overflow of norepinephrine.

Daily electroshock and daily treatment with desmethylimipramine both produced down regulation of similar magnitude of β-adrenoceptors (Table 2). Both of these chronic treatments produced modest sustained increases in basal norepinephrine overflow in the frontal cortex and hippocampus. With electroshock treatment, both the first shock and the last shock produced significant increments above the immediately preceding baseline for norepinephrine overflow (Table 1) while the first but not the tenth administration of desmethylimipramine produced a statistically significant increase over basal release (Fig. 2). These results suggest that both modest sustained increments in extracellular norepinephrine and larger acute daily elevations can produce down regulation of β-adrenoceptors.

The importance of increased extracellular norepinephrine for the down regulation of β-adrenoceptors is further demonstrated in the experiments in which daily electroshock treatments were administered to animals which had been pretreated with either reserpine or 6-hydroxydopamine to prevent the release of norepinephrine. Both of these pretreatments reduced the extracellular norepinephrine concentration such that they were below detection limits. Similarly, in the presence of pretreatment with reserpine or 6-hydroxydopamine, electroshock-induced seizures failed to increase extracellular levels of norepinephrine. In the absence of increments in extracellular norepinephrine, down regulation of β-adrenoceptors did not occur either in the frontal cortex or in the hippocampus (Table 2). In confirmation of many earlier studies, our results showed that depletion of norepinephrine by treatment with either reserpine or 6-hydroxydopamine causes up regulation of β-adrenoceptors in the frontal cortex but not in the hippocampus. The importance of extracellular norepinephrine in the down regulation of β-adrenoceptors and coupled adenylate cyclase was first suggested almost 20 years ago in experiments with 6-hydroxydopamine treated animals and animals with selective lesions of brain noradrenergic neurons (Wolfe et al., 1978; Schweitzer et al., 1979; Janowsky et al., 1982). More recently the cellular mechanisms by which extracellular norepinephrine regulates β-adrenoceptor density and sensitivity through cyclic AMP-dependent protein kinase and the β-adrenoceptor kinase have been well described (Nalepa et al., 1998).

In contrast, our findings suggest that extracellular norepinephrine is not important for the up regulation α-adrenoceptors. As noted previously, both electroshock treatment and treatment with desmethylimipramine increase extracellular norepinephrine in the frontal cortex and the hippocampus (Table 1, Figs. 1 and 2). Yet, desmethylimipramine treatment did not induce changes in B_{max} for α-adrenoceptors either in the frontal cortex or the hippocampus (Table 3). Also, electroshock produced an increment in B_{max} for α-adrenoceptors in the frontal cortex but did not produce a significant change in B_{max} in the hippocampus, even though extracellular norepinephrine was elevated in this structure. Depletion of norepinephrine with either reserpine or 6-hydroxydopamine did not prevent the electroshock-induced up regulation of α-adrenoceptors in the frontal cortex. The electroshock-induced increase in B_{max} in non-depleted animals is similar in magnitude to the increase in B_{max} seen in reserpine pretreated and 6-hydroxydopamine pretreated animals. Taken together these data strongly suggest that electroshock produces up regulation of α-adrenoceptors in a manner independent of norepinephrine release. Since 6-hydroxydopamine produces neurotoxic deletion of noradrenergic neu-

rons, the experiments that employed this agent suggest that α-adrenoceptor up regulation by electroshock is produced by a process which is entirely independent of noradrenergic neurons. A similar conclusion has been reached by Blendy et al. (1991) who suggest that chronic electroshock might affect the transcription, translation, posttranslational processing or turnover of α_1-adrenoceptor through processes which are independent of any effects on noradrenergic neurons.

In our present study, chronic desmethylimipramine treatment did not alter α_1-adrenoceptor B_{max} or K_d either in the frontal cortex or the hippocampus. Nevertheless, the treatment did produce substantial decrements in β-adrenoceptor B_{max} in both of these brain areas, an effect that is consistent with previous observations (Saraiya et al., 1978; Riva and Creese, 1989b). Stockmeier et al. (1987) and colleagues have reported the absence of an effect of chronic norepinephrine reuptake inhibitors on α_1-adrenoceptors. In contrast, some other investigators have reported an increase in α_1-adrenoceptors following such treatment (Vetulani et al., 1983; Maj et al., 1985). The observations in the current study support the concept that norepinephrine increments in extracellular fluid which are sufficient to cause down regulation of β-adrenoceptors, fail to cause changes in α_1-adrenoceptors.

Taken together the data in this current study support the concept that down regulation of β-adrenoceptors is mediated through increments in extracellular norepinephrine. The receptor regulation process appears to be independent of the means through which the increment in extracellular norepinephrine is induced. In contrast, up regulation of α-adrenoceptors appears to be independent of norepinephrine release and does not require the presence of noradrenergic neurons in order to be induced by electroshock.

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