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Effect of repetitive electroconvulsive treatment on sensitivity to pain and on [^{3}H]nitrendipine binding sites in cortical and hippocampal membranes

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Abstract. The effect of electroconvulsive shock (ECS) on the responsiveness to pain (measured by the hot-plate test) and on the characteristics of L-type calcium channels (measured as [^{3}H]nitrendipine binding sites) in the cortex and hippocampus was tested on the Wistar rat. In animals receiving a single ECS, the calcium channel density and affinity 24 h after treatment did not differ from the controls; the response to pain was also at the control level. Repeated ECS (eight once-daily shocks) resulted in an increased responsiveness to pain (shortening of response latency) and in an increase in the density of cortical, but not hippocampal, calcium channels. The K_D value for [^{3}H]nitrendipine binding sites in either brain region remained unaltered by ECS. The calcium channel antagonist nifedipine, which by itself did not significantly alter the response to pain, prevented the enhancement of pain sensitivity brought about by ECS. The results suggest activation of calcium-dependent mechanisms by repeated ECS and confirm the involvement of calcium channels in pain mechanisms.

Key words: Electroconvulsive treatment – [^{3}H]nitrendipine binding – Calcium channels – Hot plate test

Repeatedly administered electroconvulsive shock (ECS) is regarded as an efficient treatment of endogenous depression (Fink 1979). ECS has considerable pharmacological specificity and profoundly affects many neurotransmitter systems in the brain, producing alterations both in neurotransmitter content and turnover and in characteristics of several types of receptors (Gleiter and Nutt 1989). This is reflected by the fact that repeated ECS influences responses to drugs affecting some neurotransmitter systems, e.g. it attenuated responses to clonidine (Heal et al. 1981; Pilc and Vetulani 1981; Vetulani et al. 1983) and potentiated the responses to drugs activating dopamine (Green 1980; Wielosz 1981; Green et al. 1983) and serotonin (Green 1980; Vetulani et al. 1981, 1983; Green et al. 1983; Vetulani 1984) receptors. The changes in responses to drugs affecting particular neurotransmitter systems are, in general, well correlated with receptor changes induced by repeated ECS (see Vetulani 1984; Gleiter and Nutt 1989). In many respects these effects resemble those evoked by antidepressant drugs (e.g. β- or α_2-adrenoceptor downregulation), but in some cases the effects of ECS are different (e.g. action on serotonin receptors) (see Vetulani 1984, for review).

In contrast to its profound neurochemical effects, repeated ECS does not produce evident long-lasting changes in general behavior of drug-free laboratory animals, apart from the period immediately following the shock. We observed increased irritability and locomotor activity in some, but not all rats after repeated ECS (unpublished observations) and an increase in post-decapitation convulsions (Vetulani 1984). Searching for the existence of other, less apparent alterations of responsiveness of ECS-treated rats to environmental stimuli, we investigated the changes in the nociceptive response in the hot-plate test (Woolfe and MacDonald 1944). The preliminary results indicated that repeated ECS shortened the latency of the response.

The responsiveness to pain is usually linked with the opioid system. Our earlier experiments (Antkiewicz-Michaluk et al. 1984) demonstrated that chronic ECS affects the central opioid receptors, but the change, an increased density of δ receptors, does not seem to be compatible with the observed increase in responsiveness to pain.

However, pain is also related to calcium-dependent mechanisms. Calcium seems to play an important role in nociception and in the action of opioids (Ross and Cardenos 1979). Calcium channels in cell membranes regulate the concentration of cytosolic calcium and serve to link membrane potential changes with cellular responses (Reuter et al. 1982; Miller 1987). Inhibition of these channels potentiates analgesic effects of opioids and opioid-mediated stress analgesia (Harris et al. 1975; Chapman and Way 1982; Benedek and Szikszay 1984;

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Hoffmeister and Tettenborn 1986; Kavaliers and Ossenkopp 1987; Kavaliers 1987a, b), while calcium ionophores exert an opposite effect (Hoffmeister and Tettenborn 1986; Kavaliers 1987b; Kavaliers and Ossenkopp 1987). We investigated, therefore, if repeated ECS affects calcium channels, whose functional state may be measured by specific binding of [^{3}H]nitrendipine (Ramkumar and El-Fakahany 1986).

Although the effects of ECS on several receptors in cerebral membranes were investigated, the data on the effect of repeated ECS on dihydropyridine binding sites, which represent functional L-type calcium channels (Belleman et al. 1981; Middlemiss 1985; Middlemiss and Spedding 1985) are scarce and controversial. While Bolger et al. (1987) reported an increase in cortical, and a decrease in hippocampal [^{3}H]nitrendipine binding site density in the rat, and a similar trend in the cat, Gleiter et al. (1988) did not see any change in binding of either [^{3}H]nitrendipine or [^{125}I]ω-conotoxin GVIA (a specific ligand for calcium N-type channels, Thayer et al. 1988) in various brain areas of the rat.

Our results indicate that repeated, but not a single ECS may increase both the responsiveness of the rat to pain and the density of cortical [^{3}H]nitrendipine binding sites, and that the proalgesic action of ECT is inhibited by a calcium channel blocker, nifedipine.

Materials and methods

Animals and treatment

The experiments were carried out on male Wistar rats, 220–240 g, kept under standard laboratory conditions, ten to a home cage (large plastic cage with wire lid and sawdust bedding), with free access to standard laboratory food and tap water, at room temperature (approximately 22° C), in a natural day-night cycle (summer/autumn). The experiments were carried out between 09.00 and 11.00 hours.

Electroconvulsive shock treatment (ECS) was given without anesthesia by passing a current (sinusoidal, 150 mA, 50 Hz, 500 ms) through ear-clip electrodes. This procedure invariably produced convulsions lasting over 15 s. Repeated ECS consisted of eight electroshocks delivered at 24-h intervals (48 h between the sixth and seventh shocks). The controls were handled similarly, but without passing current through the electrodes. The animals were tested behaviorally or killed by decapitation 24 h after the single shock or after the last shock in the series.

Drug treatment. Approximately 24 h after the last ECS (or sham ECS), 15 min before the behavioral test, the rats were injected with 5 mg/kg IP nifedipine (Polfa). The controls received saline, 4 ml/kg IP.

Behavioral tests. The responsiveness of rats to a painful stimulus was assessed using the hot-plate test (Woolfe and MacDonald 1944). The rats were placed on a metal plate kept at 56° C and the latency of response (licking of hind paws or jumping) was measured. The cut-off time was 30 s.

Membrane preparation and receptor binding assay

The brain was rapidly removed, placed on an ice-chilled porcelain plate, and the cerebral cortex and hippocampus were dissected. The tissues were homogenized using a Polytron disintegrator (setting 4, 10 s) at 0° C in 20 vol 50 mmol/l TRIS-HCl buffer, pH (at 23° C) 7.6. The cortex from each animal was homogenized separately; the hippocampal tissue from two animals was pooled for membrane preparation.

The homogenate was centrifuged at 0° C and 100 *g* for 10 min, the supernatant was decanted and recentrifuged at 0° C and 25000 *g* for 30 min, and the resulting pellet was resuspended in the buffer and recentrifuged under the same conditions. The pellet thus obtained (fraction P_2 of Whittaker and Barker 1972) was stored at −18° C for not longer than 48 h. For incubation it was reconstituted in the TRIS-HCl buffer to obtain a final protein concentration (measured according to Lowry et al. 1951) of approximately 1.2 mg/ml.

The radioligand, [^{3}H]nitrendipine (NEN, specific activity 78.3 Ci/mmol), was prepared in six concentrations (final concn 0.04–2 nM) in the buffer. The incubation mixture (final volume 550 µl) consisted of 450 µl membrane suspension, 50 µl of a [^{3}H]nitrendipine solution and 50 µl buffer without (total binding) or with (unspecific binding) nifedipine (final concn 10 µM).

The incubation was carried out in duplicate, in a shaking water bath, at 25° C for 30 min. Addition of the radioligand initiated the incubation, which was terminated by vacuum-assisted filtration through GF/C Whatman fiberglass filters. The filters were then rinsed twice with 5 ml portions of ice-cold incubation buffer and placed in plastic scintillation minivials. Bray's fluid (3 ml) (Bray 1960) was added and the samples were counted for radioactivity in a Beckman LS 3801 scintillation counter.

The specific binding was defined as the difference between total and unspecific binding, and was expressed in fmol/mg protein. The results were evaluated by Scatchard analysis for assessment of B_{max} and K_D values.

Statistics

The significance of differences was assessed with one way analysis of variance, followed, if required, by the Tukey-Kramer test.

Results

Hot-plate test

Animals tested 24 h after a single ECS had a similar hot plate latency to the controls, while the latency of those subjected to repeated ECS was significantly shortened by approximately 35%. Nifedipine did not significantly prolong the hot-plate latency in control animals (subjected to a single sham ECS), but prevented the decrease in the latency in rats that had received repeated ECS (Table 1).

Table 1. The effect of ECS and a calcium channel blocker on hot-plate latency

Treatment	Latency (s)	% of control
Control, single	8.1 ± 0.64 (20)	100
ECS, single	8.0 ± 0.54 (10)	99
Nifedipine, single	9.2 ± 0.90 (9)	114
Control, chronic	8.8 ± 0.78 (9)	100
ECS, chronic	5.6 ± 0.61 (14)	64*
ECS, chronic + nifedipine	8.0 ± 0.58 (15)	91

The tests were carried out 24 h after a single or the last of a series of ECS. Nifedipine was given only once, 15 min before the test. The data are means ± SEM (*N*). For single treatment experiments $F = 0.67$ (df 2/36), for chronic experiments $F = 6.54$ (df 2/35; $P < 0.01$). * $P < 0.01$ (Tukey-Kramer test)

Table 2. The effect of ECS on specific [^{3}H]nitrendipine binding to cerebral membranes

	Cortex			Hippocampus		
	N	B_{max} (fmol/mg prot)	K_D (nM)	*N*	B_{max} (fmol/mg prot)	K_D (nM)
Single treatment						
Control	16	95±7	0.86±0.05	4	126±6†	0.77±0.14
ECS	10	96±6	0.68±0.05	4	123±9	0.69±0.07
% control		101	79		98	90
Chronic treatment						
Control	8	106±4	0.65±0.04	4	143±13†	0.74±0.04
ECS	8	136±9	0.70±0.04	4	126±10	0.62±0.04
% control		128*	116		88	84

The data are means±SEM. *N* – number of independent Scatchard plots (6 concentrations, in duplicate). Rats killed 24 h after the last ECS. Chronic ECS – 8 shocks. * $P<0.05$ (difference from control), † $P<0.01$ (difference from cortex)

[^{3}H]Nitrendipine binding

In the concentration range used, the Scatchard plots for [^{3}H]nitrendipine binding were in all cases rectilinear (*r* coefficient ranging from 0.87 to 0.95), suggesting homogeneity of [^{3}H]nitrendipine binding sites.

In control rats the density of [^{3}H]nitrendipine binding sites in the hippocampus was higher than in the cortex by 35% ($P<0.01$), while the K_d values for those sites in both brain areas were similar (Table 2).

The density of [^{3}H]nitrendipine binding sites in the cortical membranes was significantly higher (by approximately 30%) in rats receiving repeated ECS, but the K_d values remained unchanged. In the hippocampus the parameters of [^{3}H]nitrendipine binding sites were not affected significantly by repeated ECS; a non-significant 12% decrease of B_{max} was noted (Table 2).

Discussion

The present results indicate that repeated ECS, given in a manner which is regarded as a model mimicking the clinical application and which produces several adaptive receptor changes in the cerebral cortex of the rat (see Vetulani 1984), also increases the responsiveness to pain. The treatment also results in a specific increase in the density of [^{3}H]nitrendipine binding sites, suggesting an increase in the number of functional cortical L-type calcium channels, for which nitrendipine shows high affinity (Nowycky et al. 1985). Blockade of calcium channels with a calcium antagonist, nifedipine, shortly before the test did not significantly affect the nociceptive response in control rats, but abolished the effect of ECS treatment. These results suggest that the increased responsiveness to pain after chronic ECS is caused by an increased calcium availability for the neuron, resulting from an increase in the density of calcium channels.

The results concerning [^{3}H]nitrendipine binding confirm those of Bolger et al. (1987); even the insignificant depression in the density of hippocampal [^{3}H]nitrendipine binding sites is quantitatively very similar to a significant 11% depression reported by them. As the experiments were carried out on rats of different strains, they suggest that the action of ECS on calcium channels is not strain specific, unlike some other receptor effects of ECS, e.g. on α_1- and β-adrenoceptors (Schulz and Kopansky 1985; Vetulani et al. 1986).

A question arises as to whether the observed increase in the density of calcium channels may be related to specific changes in density of other receptors brought about by ECS. The increase in responsiveness to nociceptive stimuli suggest a link with ECS-induced elevation of opioid receptors in the cortical membranes (Antkiewicz-Michaluk et al. 1984; Holaday et al. 1986). It has been observed that morphine dependence (Ramkumar and El-Fakahany 1988) and abstinence (unpublished observations) is associated with an increase in the density of [^{3}H]nitrendipine binding sites.

Another possibility is that an increase in the density of calcium channels is a compensatory response to inhibition of calcium current evoked by various neurotransmitters, which are massively released during ECS. Although the mechanism of inhibition is disputable, earlier studies suggesting a decrease in the number of calcium channels (Dunlap and Fischbach 1981; Galvan and Adams 1982; Forscher and Oxford 1985) while a recent one suggested a change in voltage dependence of channels (Bean 1989), in either case a reduction in calcium current may be counteracted by increasing the number of functional calcium channels.

It is at present difficult to postulate a role for changes in the density of a particular type of calcium channels for antidepressant action of ECS. However, it might be speculated that an increase in excitatory responses to several psychotropic agents and neurotransmitter agonists observed after this treatment and ascribed to its antidepressant action may result from an increased calcium influx into the cerebral neurons.

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