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Effects of Single and Repeated Electroconvulsive Shock on Isoproterenol-Stimulated Pineal *N*-acetyltransferase Activity and Melatonin Production in Rats

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MONTELEONE, P., L. STEARDO, M. D'ISTRIA, I. SERINO AND M. MAJ. *Effects of single and repeated electroconvulsive shock on isoproterenol-stimulated pineal* N*-acetyltransferase activity and melatonin production in rats.* PHARMACOL BIOCHEM BEHAV **50**(2) 241-244, 1995.—The response of the pineal gland to acute isoproterenol administration represents a useful tool to investigate β_1-adrenoceptor function, because the production of melatonin and the activity of its main synthesizing enzyme, *N*-acetyltransferase (NAT), are regulated by β_1-adrenergic receptors. In the present study, rats underwent single electroconvulsive shock (ECS) administration (0.80 mA, 0.5 s, at midday), chronic ECS treatment (0.80 mA, 0.5 s, once daily for 8 days), or sham treatments. On the day after the last ECS or sham ECS, animals were injected with isoproterenol hydrochloride (1 mg · kg^{-1} SC) or volume-matched saline at 1600 h. After single ECS, isoproterenol injection induced a clear-cut increase in both pineal NAT activity and melatonin levels with no significant differences between ECS-treated rats and the sham-treated ones. In rats chronically treated with ECS, the isoproterenol-induced increases in both pineal NAT activity and melatonin content were significantly lower than in sham-treated animals ($p < 0.001$ for NAT activity; $p < 0.005$ for melatonin levels; Tukey's test). These data show that the pinealocyte β-adrenoceptor function is reduced by chronic, but not acute ECS administration, and that this change is not due to the nonspecific stress effect of animal handling or to the acute effects of the last of a series of ECS.

β-Adrenoceptors Electroconvulsive shock Isoproterenol Melatonin NAT Pineal gland

ELECTROCONVULSIVE therapy (ECT) has played a central role in psychiatric treatment for almost 50 yr. Much recent research on its mechanism of action has stressed alterations in brain receptor sensitivity occurring in rats after repeated electroconvulsive shock (ECS), which is considered to be an animal model of ECT (4).

In particular, the downregulation of cortical β-adrenergic receptors has been the most widely reported change occurring after both chronic ECS and long-lasting treatment with different antidepressant drugs (2,8,16,19). Hence, it has been suggested that β-adrenergic receptors may be involved in the therapeutic mechanism of both ECT and antidepressant compounds. In addition, recent studies have suggested that chronic ECS treatment as well as chronic antidepressant drug administration downregulate β_1- but not β_2-adrenoceptors in the rat brain (9).

In rats, the secretion of melatonin by the pineal gland is regulated mainly by norepinephrine, which acts on β_1-

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adrenergic receptors localized on the pinealocytes, and it is widely demonstrated that the IP injection of isoproterenol, a specific β-adrenoceptor agonist, stimulates pineal melatonin production (18). Therefore, in both experimental animals and humans, the pineal gland has been suggested to be an appropriate neuroendocrine model to investigate the status of adrenergic function (17).

We recently showed that chronic ECS treatment blunts the increase in rat melatonin levels induced by isoproterenol (13), and that seizure activity is indeed required to induce this change (14). These data allowed us to suggest that chronic ECS may affect the functional status of pineal β_1-adrenergic receptors, possibly through their downregulation.

According to Grahame-Smith et al. (7), neurochemical ECS-induced effects in the rat brain have greater relevance to clinical ECT mechanisms if they are demonstrable after chronic but not single ECS. Therefore, in the present study, we aimed to assess the rat pineal response to isoproterenol after acute ECS treatment. Moreover, because, in the rat, a strict correlation exists between pineal melatonin content and the activity of its main synthesizing enzyme, the *N*-acetyltransferase (NAT) (17), we evaluated whether ECS-induced changes in the isoproterenol-stimulated melatonin production are associated to concomitant alterations in the NAT activity.

METHOD

Male Sprague–Dawley rats (n = 40), weighing 300–350 g, were used in the experiments. They were housed four to five per cage in a temperature-controlled environment (22 ± 2°C) with a 12 h : 12 h light–dark cycle (lights on at 0600 h). Animals were allowed free access to food and water.

Single ECS (80 mA, 0.5 s) was administered via ear-clip electrodes and without anesthesia. This procedure always resulted in a generalized tonic-clonic seizure lasting 20–30 s, followed by a brief (1–2 min) period of postictal stupor, with full recovery within a few minutes. Control animals received sham ECS, which involved identical handling procedures with application of ear-clip electrodes but no current. ECS was administered between 1130 and 1200 h.

Acute Experiment

After one adaptation week, 10 rats received single ECS and 10 rats sham ECS. On the day after the ECS or sham-ECS treatment, five rats of each group were injected with isoproterenol hydrochloride (1 mg · kg^{-1} SC) at 1600 h. The remaining 10 animals (five ECS-treated and five sham-treated) were injected with a volume-matched saline at the same time. After 2 h (at 1800 h), animals were decapitated, one per group as a sequence without interruption so that no difference in the time of killing occurred among the groups.

Chronic Experiment

After one adaptation week, 10 rats underwent ECS once per day for 8 days and 10 rats received sham ECS. On the day after the last ECS or sham ECS, five rats of each group were injected with isoproterenol hydrochloride (1 mg · kg^{-1} SC) at 1600 h. The remaining 10 animals (five ECS-treated and five sham-treated) were injected with a volume-matched saline at the same time. After 2 h (at 1800 h), animals were decapitated, one per group as a sequence without interruption so that no difference in the time of killing occurred among the groups.

In both the acute and chronic experiments, rats were injected with isoproterenol on the day after the single or the last ECS to exclude possible nonspecific stress effects of the ECS treatment on the pineal activity.

The pineal glands were rapidly dissected, frozen on solid CO_2, and stored at −80°C until processed for melatonin and NAT assay. Pineal glands were homogenized in 0.05 M phosphate buffer, pH 7.4. Aliquots (10 μL) were used to measure NAT activity by the radioenzymatic method of Champney et al. (3). Each sample was incubated for 20 min at 37°C in the presence of tryptamine-HCl (Sigma Chemical Co., St. Louis, MO), acetyl-coenzyme A (Sigma Chemical Co.), and acetyl-(1-^{14}C)-coenzyme A (Amersham International plc, Amersham, UK; sp act: 60 mCi/mmol; final specific activity in the incubation mixture: 17 mCi/mmol). Results were expressed as nmoles of *N*-acetyltryptamine formed per gland per hour.

For melatonin assay, homogenate aliquots of 50 μL were extracted by diethylether, and pineal melatonin levels were determined by radioimmunoassay (1), using a sheep melatonin antiserum from Guildhay Antiserum (University of Surrey, UK), [^{3}H]melatonin tracer (Amersham, Bucks, UK; sp act: 85 Ci/mmol), and unlabeled melatonin from Sigma Chemical Co. Free and antibody-bound fractions of [^{3}H]melatonin were separated using a dextran-coated charcoal solution (2% : 0.2%). The lower and the upper detection limits of the assay were 6 and 200 pg/ml, respectively. Intra- and inter-assay coefficients of variation were 5.1 and 9.8%, respectively.

Results are expressed as mean ± SD and were statistically analyzed by two-way analysis of variance (ANOVA) and Tukey's test.

RESULTS

Acute Experiment

Compared with saline, isoproterenol administration resulted in a clear-cut increase in both pineal NAT activity ($p < 0.001$, Tukey's test) and melatonin content ($p < 0.001$), with no significant differences between ECS-treated rats and sham-treated ones (Fig. 1). Two-way ANOVA showed no significant effect for shock treatment [$F(1, 16) = 0.309$ for melatonin, and $F(1, 16) = 0.830$ for NAT activity], a significant effect for drug treatment [$F(1, 16) = 194.6$; $p < 0.00001$ for melatonin, and $F(1, 16) = 93.98$; $p < 0.00001$ for NAT], and no significant shock × drug interaction [$F(1, 16) = 0.086$ for melatonin, and $F(1, 16) = 0.907$ for NAT activity].

Chronic Experiment

The effects of isoproterenol administration on pineal NAT activity were significantly different in the shock-treated rats compared with the sham-treated ones. Indeed, with respect to saline, isoproterenol administration resulted in a clear-cut increase of NAT activity in both sham-treated rats ($p < 0.001$) and ECS-treated ones ($p < 0.001$). However, in animals that received chronic ECS, the isoproterenol-induced increase in pineal NAT activity was significantly lower than in those undergoing sham procedures ($p < 0.001$, Tukey's test) (Fig. 2, top panel). Two-way ANOVA disclosed significant effects for shock treatment [$F(1, 16) = 6.373$; $p = 0.02$] and for drug treatment [$F(1, 16) = 33.798$; $p < 0.00001$], and a significant shock × drug interaction [$F(1, 16) = 6.333$; $p = 0.02$].

For pineal melatonin levels in ECS-treated rats, the effects of isoproterenol administration on pineal melatonin levels were significantly different in the shock-treated animals compared with sham-treated ones. In fact, compared with saline,

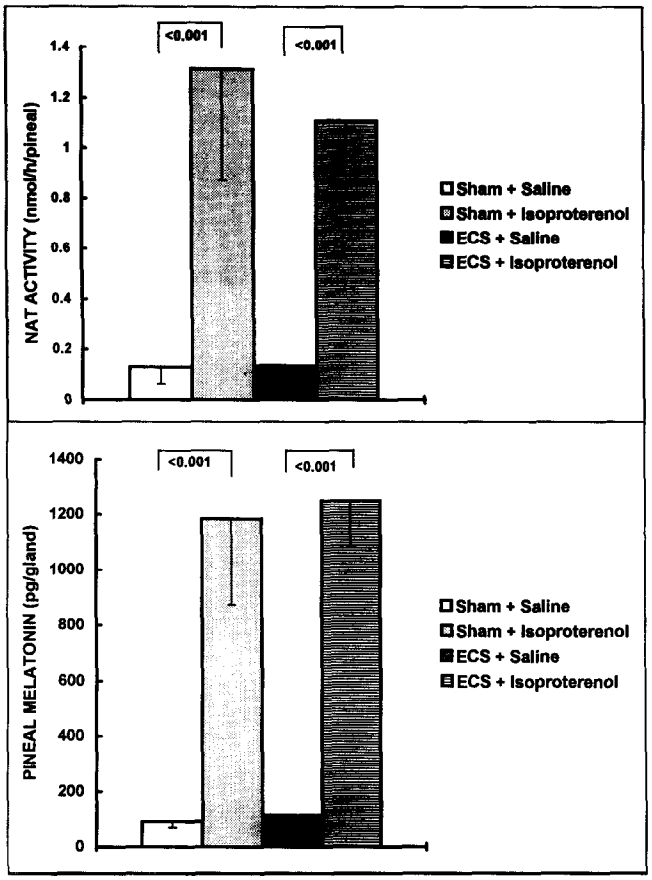

FIG. 1. Pineal NAT activity (top) and melatonin levels (bottom) after saline or isoproterenol (1 mg · kg^{-1} SC) administration in rats treated with acute electroconvulsive shock (ECS) (80 mA, 0.5 s) or sham ECS. Data are expressed as mean ± SD.

isoproterenol significantly increased pineal melatonin content in both sham-treated animals ($p < 0.001$) and ECS-treated ones ($p < 0.001$). However, in animals that received chronic ECS, the isoproterenol-induced increase in pineal melatonin levels was significantly lower than in those undergoing sham procedures ($p < 0.005$) (Fig. 2, bottom panel). Two-way ANOVA showed significant effects for shock treatment [$F(1, 16) = 5.049$; $p = 0.03$] and for drug treatment [$F(1, 16) = 61.571$; $p < 0.00001$], and a significant shock × drug interaction [$F(1, 16) = 4.769$; $p = 0.04$].

DISCUSSION

Our present results confirm our previous observations (13,14) that chronic ECS administration decreases the melatonin production induced by isoproterenol in rats. Moreover, this study shows for the first time that the blunting effect of ECS on isoproterenol-stimulated melatonin synthesis occurs after chronic but not acute ECS administration, and that it is associated with a concomitant decrease in the activity of the NAT enzyme.

A previous report on pineal melatonin content after both acute and chronic ECS in rats showed no effect of electrically induced seizures on pineal activity, as assessed by a single measurement of unstimulated melatonin levels (12). Our data confirm that both acute and chronic ECS do not affect the basal activity of the rat pineal gland as shown by both NAT values and melatonin levels in saline-injected animals. However, when the activity of the gland was evaluated as a response to β_1-adrenergic stimulation, our study shows a clear-cut difference between acute and chronic ECS treatments, because isoproterenol-induced rises of both NAT activity and melatonin content were blunted in chronically, but not acutely shocked rats.

It is well known that in the rat, the stimulation of β_1-adrenergic receptors on the pinealocytes leads to the activation of the membrane-bound adenylate cyclase, with a consequent increase in the intrapinealocyte c-AMP levels. This, in turn, stimulates the NAT enzyme, producing an increase of pineal melatonin synthesis (18). The present findings demonstrate that the blunting effect of chronic ECS on isoproterenol-induced melatonin synthesis is related to a reduced activity of its main synthesizing enzyme (i.e., NAT). Consistent with this observation, Friedman et al. (6) demonstrated that chronic, but not acute treatment with antidepressant drugs, such as imipramine or iprindole, blunted the darkness-induced increase in both pineal melatonin levels and NAT activity.

In addition, other authors have shown that in the rat, chronic administration of various antidepressant drugs signifi-

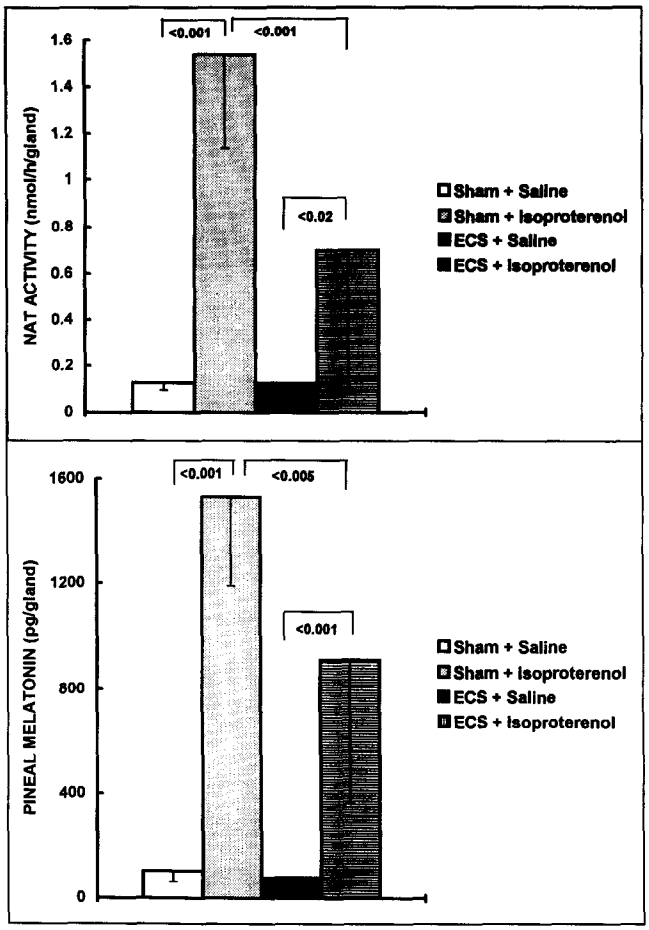

FIG. 2. Pineal NAT activity (top) and melatonin levels (bottom) after saline or isoproterenol (1 mg · kg^{-1} SC) administration in rats treated with chronic electroconvulsive shock (ECS) (80 mA, 0.5 s; once daily for 8 days) or sham ECS. Data are expressed as mean ± SD.

cantly reduces both the number of pinealocyte β_1 adrenoceptors and c-AMP or melatonin responses to noradrenergic stimulations (5,10,15). These data suggest that long-lasting antidepressant drug-treatments actually downregulate β_1-adrenergic receptors in the rat pineal gland as they do in the CNS. Hence, because parallels between ECS and antidepressant drug effects on brain noradrenergic function have been consistently reported (11), it seems likely that in our study, repeated ECS affected the noradrenergic modulation of pineal activity possibly through a downregulation of pinealocyte β_1-adrenergic receptors. Probably, the direct assessment of pineal β_1-adrenoceptor number and affinity would have been the more appropriate method to give direct evidence of their downregulation after chronic ECS. Therefore, because we did not perform such an analysis, we cannot exclude that postreceptor mechanisms might be involved in the blunting effect of chronic ECS treatment on pineal responsivity to isoproterenol.

In conclusion, our results show that in the rat, the pineal response to β-adrenergic stimulation is decreased after chronic, but not acute ECS administration. This change does not result from nonspecific handling effects of the animals or from the acute effects of the last of a series of ECS. The relevance of these findings to the therapeutic mechanism of ECT remains to be determined.

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