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Beta-1 adrenoceptor antagonists potentiate the anticonvulsive effect of swim stress in mice

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Abstract

To explore the possible involvement of beta adrenoceptor antagonists in the previously observed anticonvulsive effect of swim stress, the mice were, prior to administration of convulsants, pre-treated with propranolol (a non-selective beta adrenoceptor antagonist), betaxolol (a selective beta-1 adrenoreceptor antagonist), or ICI 118,551 (a selective beta-2 adrenoreceptor antagonist). In control unstressed animals, only propranolol [10 mg/kg, intraperitoneally (ip)] produced a significant change. It enhanced the threshold dose of picrotoxin producing tonic hindlimb extension. However, in swim-stressed animals, propranolol enhanced doses of picrotoxin producing tonic hindlimb extension and death, while betaxolol (20 mg/kg, ip) enhanced doses of picrotoxin producing running/bouncing clonus, tonic hindlimb extension and death. Pre-treatment with ICI 118,551 (4 mg/kg, ip) failed to affect doses of picrotoxin producing convulsions and death. The results demonstrate that blockade of beta-1 adrenoceptors potentiates the anticonvulsant effect of swim stress against convulsions produced by picrotoxin, a noncompetitive $GABA_A$ receptor antagonist.

Keywords: Swim stress; Convulsions; Mice; Picrotoxin; Propranolol; Betaxolol; ICI 118,551

Various studies have shown that stressful manipulations in rats and mice lower the convulsant potency of GABA-related convulsants [1–3,7,8,11]. The anticonvulsive effect of stress could not be counteracted by the antagonist of benzodiazepine binding sites — flumazenil [7], by adrenalectomy [8], or drugs interfering with the synthesis of steroids [9]. On the other hand, it has been suggested that beta adrenoceptors are involved in stress-related behaviors in both rats and mice [5]. Beta-1 adrenoceptor antagonists have been shown either to potentiate and imitate the behavioral effects of stress [12–14], or to block the effects of stress on some forms of behavior [15].

Hence, the aim of this study was to explore whether beta-1 adrenoceptor antagonists interfere with the effects of swim stress on the convulsant signs and death produced in male CBA mice by intravenous (iv) infusion of picrotoxin, a non-competitive $GABA_A$ receptor antagonist.

1. Method

1.1. Animals

Male CBA mice (25–30 g), 3 months old, were used. They were housed at a constant temperature (22°C) and with a light cycle of 12 h light/12 h darkness (lights on at 7:00 AM). They were caged in groups of 10. Food and water were freely available. Prior to experiment, the animals were not habituated either to intraperitoneal or intravenous drug administration. The procedures used in the study were in compliance with the European Communities Council Directive of 24 November 1986 (86/609/EEC).

1.2. Stress procedure

Mice were subjected to swim stress (10-min swimming at 18–19°C). The swimming tank (28 cm diameter, 25 cm height) was filled up with water 15 cm high. After swimming, the animals were dried by a towel and placed near the heater. The intravenous injection of picrotoxin started 15 min after termination of stress.

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1.3. Drugs

Picrotoxin and ICI 118,551 (Sigma, St. Louis, MO), betaxolol (Synthelabo, Bagneux, France) and *S*(−)-propranolol HCl (RBI, Natick, MA) were used. All drugs were dissolved in saline. Picrotoxin was given by constant intravenous infusion into a tail vein, while other drugs were administered intraperitoneally (ip) in a volume 1 ml/100 g body weight, 35 min before swim stress and 1 h before starting infusion of picrotoxin. Control animals received the corresponding vehicles.

1.4. Convulsive activity

For the determination of convulsive activity, the animal was taken from its home cage and placed in a glass cylinder (20 × 7 cm) with numerous holes for ventilation. The tail of the animal was drawn through a hole of the plastic cover, and warmed for 1 min under a tensor lamp. A butterfly infusion needle (0.3 mm) was inserted into the tail vein and correct placement was verified by the appearance of blood in the infusion tubing. During the infusion, the animal was held lightly by the tip of the tail to allow free movement. The concentrations of picrotoxin was 0.75 mg/ml, and the infusion rates were 0.55 ml/min.

The animal was observed throughout infusion and the time between the start of infusion and the onset of convulsive signs, mainly as described by Kosobud and Crabbe [6], was measured. The convulsive signs were: running/bouncing clonus (a violent whole-body clonus, including running and explosive jumps) and tonic hindlimb extension (characterised by extreme rigidity, with forelimbs and hindlimbs extended caudally). For each animal, the threshold dose of convulsant (mg/kg of body weight) required to elicit a particular convulsant sign was calculated from the time of infusion, the infusion rate, the concentration of convulsant and body weight. The time to death was also measured. All experiments were carried out between 9:00 AM and 1:00 PM.

1.5. Statistical analysis

Results are expressed as mean values ± standard error of the mean (S.E.M.). Statistical analysis of the results was by one-way analysis of variance (ANOVA) followed by Newman–Keuls test and by two-way ANOVA since in the same experiment, the factors stress and drug were studied. *P* values of less than .05 were considered significant.

2. Results

As shown in Fig. 1, 15 min after exposure to swim stress (10 min swimming in water at 18–19°C), the threshold doses of picrotoxin producing running/bouncing clonus, tonic hindlimb extension and death were enhanced by

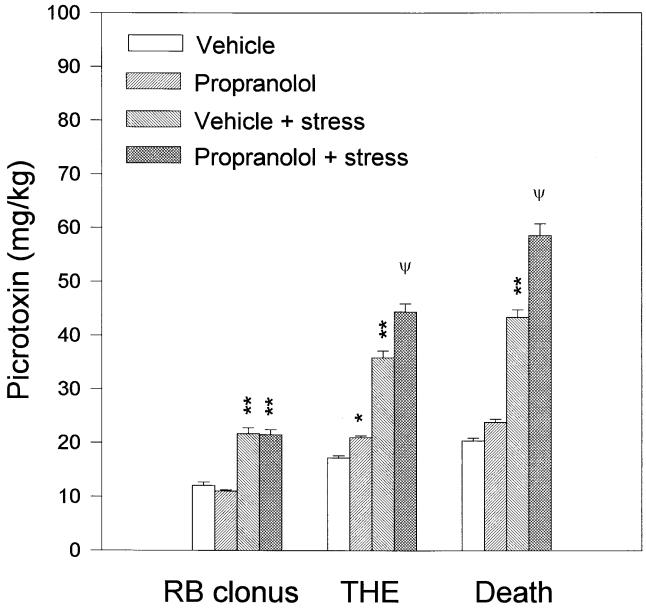

Fig. 1. Effect of propranolol and swim stress on threshold doses of picrotoxin producing running/bouncing clonus (RB clonus), tonic hindlimb extension (THE) and death in male CBA mice. Propranolol (10 mg/kg) was administered intraperitoneally 35 min prior to beginning of swim stress and 1 h prior to beginning of intravenous infusion of picrotoxin. The bars are means + S.E.M. from six to seven animals in the group. $*P<.05$ vs. the corresponding unstressed vehicle-treated group; $**P<.01$ vs. the corresponding unstressed vehicle- or (−) propranolol-treated group; $^{\Psi}P<.01$ vs. all three corresponding groups (ANOVA followed by Newman–Keuls test).

80%, 108% and 113% respectively, i.e. swim stress, as indicated by two-way ANOVA produced a highly significant ($P<.0001$) anticonvulsive effect [running/bouncing clonus: $F(1, 21)=155.06$; tonic hindlimb extension: $F(1, 20)=344.71$; death: $F(1, 21)=382.26$]. Propranolol given in a dose 10 mg/kg failed to produce a significant effect on doses of picrotoxin producing running/bouncing clonus, but the effects on tonic hindlimb extension [$F(1, 20)=31.96$] and death [$F(1, 21)=41.38$] were highly significant ($P<.0001$). Differences in the effect of propranolol on threshold doses of picrotoxin producing tonic hindlimb extension and death between unstressed and stressed animals were reflected by a significant drug × stress interaction [tonic hindlimb extension: $F(1, 20)=4.49$, $P<.05$; death: $F(1, 21)=15.25$, $P<.001$].

Planned comparisons using the Newman–Keuls test indicated that propranolol enhanced in unstressed animals only the dose of picrotoxin producing tonic hindlimb extension ($P<.05$), while in stressed animals, the same drug augmented significantly the doses of picrotoxin producing tonic hindlimb extension and death ($P<.01$ vs. all other groups).

In the experiment with betaxolol (Fig. 2.), a two-way ANOVA revealed again a profound influence of stress on threshold doses of picrotoxin producing running/bouncing

clonus [$F(1, 20) = 95.78$], tonic hindlimb extension [$F(1, 20) = 174.40$] and death [$F(1, 20) = 136.52$], $P < .0001$ for all analyses. While the effect of betaxolol (20 mg/kg) on the doses of picrotoxin producing running/bouncing clonus failed to reach the level of statistical significance [$F(1, 20) = 3.21$], the effects of drug on tonic hindlimb extension [$F(1, 20) = 24.59$] and death [$F(1, 20) = 17.69$] were highly significant ($P < .0001$ and $P < .0005$, respectively). Differences in the effect of betaxolol on stressed and unstressed animals were reflected in a significant drug × stress interaction [tonic hindlimb extension: $F(1, 20) = 13.37$, $P < 002$; death: $F(1, 20) = 9.95$, $P < .005$]. One-way ANOVA followed by Newman–Keuls test indicated that betaxolol potentiated also the effect of swim stress on doses of picrotoxin producing running/bouncing clonus ($P < .05$), but failed to modify threshold doses of picrotoxin producing convulsant signs and death in unstressed animals.

In the third experiment (Fig. 3), a two-way ANOVA revealed a highly significant ($P < .0001$) effect of stress on the threshold doses of picrotoxin producing running/bouncing clonus [$F(1, 21) = 195.70$], tonic hindlimb extension [$F(1, 22) = 107.09$] and death [$F(1, 22) = 98.60$] and a non-significant effect of ICI 118,551 given in a dose of 4 mg/kg. Planned comparisons using the Newman–Keuls test indicated only differences between the stressed and unstressed groups ($P < .001$ for all analyses).

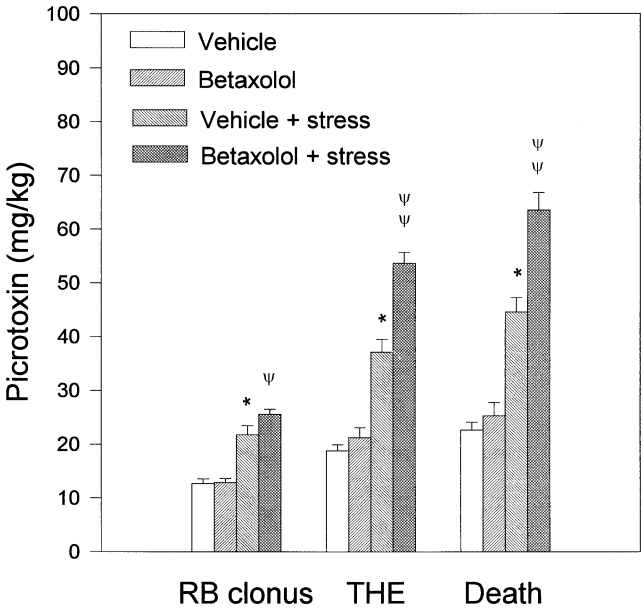

Fig. 2. Effect of betaxolol and swim stress on threshold doses of picrotoxin producing running/bouncing clonus (RB clonus), tonic hindlimb extension (THE) and death in male CBA mice. Betaxolol (20 mg/kg) was administered intraperitoneally 35 min prior to beginning of swim stress and 1 h prior to beginning of intravenous infusion of picrotoxin. The bars are means + S.E.M. from six animals per group. $^{*}P < .01$ vs. the corresponding unstressed groups; $^{\Psi}P < .01$ vs. the corresponding unstressed groups and $P < .05$ vs. the corresponding vehicle-treated stressed group. $^{\Psi\Psi}P < .01$ vs. all three corresponding groups (ANOVA followed by Newman–Keuls test).

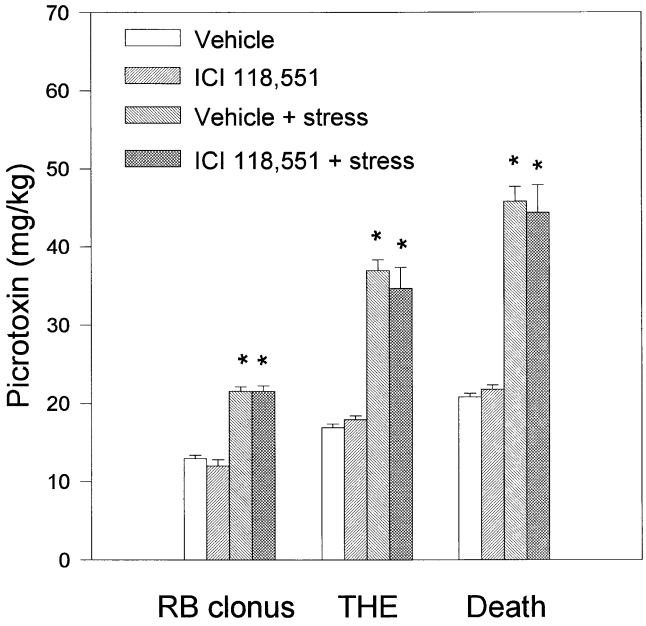

Fig. 3. Lack of effect of ICI 118,551 on swim-stress-induced enhancement of the seizure threshold for picrotoxin producing running/bouncing clonus (RB clonus), tonic hindlimb extension (THE) and death in male CBA mice. ICI 118,551 (4 mg/kg) was administered intraperitoneally 35 min prior to beginning of swim stress and 1 h prior to beginning of intravenous infusion of picrotoxin. The bars are means + S.E.M. from six to seven animals per group. $^{*}P < .01$ vs. the corresponding unstressed vehicle- or ICI 118,551-treated group (ANOVA followed by Newman–Keuls test).

3. Discussion

Stress-induced decreases in the convulsant potency of picrotoxin and other GABA-related convulsants have previously been observed [1–3,7–9,11], but the mechanisms underlying these effects remained unknown. The anticonvulsant effects of swim stress observed in the present study were as intensive as in our previous study [9], and propranolol, a non-selective beta adrenoceptor antagonist, enhanced in stressed animals by 24% the threshold dose of picrotoxin producing tonic hindlimb extension and by 35% the dose of picrotoxin producing death. In control unstressed animals, propranolol only enhanced the threshold doses of picrotoxin producing tonic hindlimb extension.

The effects of betaxolol, a selective beta-1 adrenoceptor antagonist, were in stressed animals even more pronounced than the effects of propranolol, and expressed on threshold doses of picrotoxin producing both convulsant signs and death. While in stressed animals, the observed betaxolol-induced enhancements of the threshold dose of picrotoxin producing two convulsant signs and death were 17%, 45% and 42%, respectively, in unstressed animals betaxolol failed to produce an effect. The results obtained with

betaxolol suggested that the effects of the non-selective drug propranolol obtained in stressed animals were achieved by blocking the beta-1 adrenoceptors.

A lack of effect of ICI 118,551, given in a dose supposed to selectively antagonise beta-2 adrenoceptors [12], on stress-induced enhancement of the convulsant threshold for picrotoxin indicated additionally that previously described effects are specific for the beta-1 adrenoceptor antagonists. Previous studies have demonstrated that beta-1 adrenoceptor antagonists, just like the restraint stress, delay the arousal from anesthesia [12], mimic the action of stress on motor activity in mice [13] and potentiate immobilization stress-induced changes in passive avoidance and open-field emergence tests in mice [14]. However, some other studies have shown that stress shortened pentobarbital-induced sleeping time, and propranolol reversed this effect [10]. Propranolol also attenuated restraint-induced behavioral and endocrine changes in mice [5] and inhibited stress-induced decrease in hippocampal glucocorticoid receptor mRNA levels [16]. Accordingly, although there are some inconsistencies in results, most of the studies support the proposed anxiolytic activity of beta adrenoceptor antagonists [17]. Anyway, since in our study, betaxolol was inactive in unstressed animals, and propranolol produced a marginal effect on tonic hindlimb extension only, we could not say that beta-1 adrenoceptor antagonists mimic the effect of stress. On the contrary, these drugs failed to produce an effect unless the brain homeostasis was disturbed by stress. The presented results also suggest that the pre-treatment of animals with beta-1 adrenoceptor blockers made the animals more sensitive to the effects of stress, i.e. these drugs facilitated and potentiated the anticonvulsive effect of stress.

The observed differences in drug- and stress-induced enhancements of the seizure threshold for picrotoxin between the convulsive signs are in accordance with the suggestion that the convulsive responses which appear following administration of convulsants represent three qualitatively distinct seizure components mediated by separable and independent anatomical circuits located in forebrain and hindbrain [4].

In conclusion, this study has shown that propranolol, a nonselective beta-1 and beta-2 adrenoceptor blocker, as well as a 5-HT_1 receptor antagonist, and betaxolol, a selective beta-1 adrenoceptor antagonist, potentiated the swim stress-induced enhancement of threshold doses of picrotoxin producing two convulsant signs and death in male CBA mice. In the same model, ICI 118,551, a selective beta-2 adrenoceptor antagonist, was inactive. Accordingly, the present data indicate that beta1-adrenoceptor antagonists potentiate the anticonvulsive effect of swim stress in mice. The mechanisms responsible for these effects should be further studied.

Acknowledgments

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References

[1] Abel AL, Berman RF. Effects of water immersion stress on convulsions induced by pentylenetetrazol. Pharmacol Biochem Behav 1993;45:823–5.
[2] Drugan RC, Basile AS, Ha J-H, Ferland RJ. The protective effects of stress control may be mediated by increased brain levels of benzodiazepine receptor agonists. Brain Res 1994;661:127–36.
[3] Drugan RC, McIntyre TD, Alpern HP, Maier SF. Coping and seizure susceptibility: control over shock protects against bicuculline-induced seizures. Brain Res 1985;342:9–17.
[4] Gale K. Progression and generalization of seizure discharge: anatomical and neurochemical substrates. Epilepsia 1988;29(Suppl. 2): S15–S34.
[5] Gorman AL, Dunn AJ. β-Adrenergic receptors are involved in stress-related behavioral changes. Pharmacol Biochem Behav 1993;45:1–7.
[6] Kosobud AE, Crabbe JC. Genetic correlations among inbred strain sensitivity to convulsions induced by 9 convulsant drugs. Brain Res 1990;526:8–16.
[7] Peričić D, Bujas M. Sex differences in bicuculline-induced convulsions: interaction with stress and ligands of benzodiazepine binding sites. Brain Res 1997;752:279–84.
[8] Peričić D, Bujas M, Mirković K, Jazvinšćak M. The effects of adrenalectomy and gonadectomy on sex and stress-induced differences in bicuculline-induced convulsions. Exp Brain Res 1999;129:451–6.
[9] Peričić D, Švob D, Jazvinšćak M, Mirković K. Anticonvulsive effect of swim stress in mice. Pharmacol Biochem Behav 2000;66: 879–86.
[10] Shibasaki T, Yamada K, Yamauchi N, Imaki T, Hotta M, Demura H. Beta(1)-adrenergic mechanism is involved in stress-induced increase in arousal. Neurosci Lett 1994;180:167–70.
[11] Soubrie P, Thiebot MH, Jobert A, Montastruc JL, Hery F, Hamon M. Decreased convulsant potency of picrotoxin and pentetrazol and enhanced [^{3}H]flunitrazepam cortical binding following stressful manipulations in rats. Brain Res 1980;189:505–17.
[12] Stone EA, Manavalan JS, Quartermain D. Delayed arousal from anesthesia: a further similarity between stress and beta-1 adrenoceptor blockade. Pharmacol Biochem Behav 1996;55:131–3.
[13] Stone EA, Manavalan JS, Zhang Y, Quartermain D. Beta adrenoceptor blockade mimics effects of stress on motor activity in mice. Neuropsychopharmacology 1995;12:65–71.
[14] Stone EA, Najimi M, Quartermain D. Potentiation by propranolol of stress-induced changes in passive avoidance and open-field emergence tests in mice. Pharmacol Biochem Behav 1995;51:297–300.
[15] Stone EA, Rhee J, Quartermain D. Blockade of effect of stress on risk assessment behavior in mice by a beta-1 adrenoceptor antagonist. Pharmacol Biochem Behav 1996;55:215–7.
[16] Tritos N, Kitraki E, Philippidis H, Stylianopoulou F. Neurotransmitter modulation of glucocorticoid receptor mRNA levels in the rat hippocampus. Neuroendocrinology 1999;69:324–30.
[17] Tyrer P. Anxiolytics not acting at the benzodiazepine receptor: beta blockers. Prog Neuro-Psychopharmacol Biol Psychiatry 1992;16: 17–26.