# MODULATING EFFECT OF CERULEIN ON BENZODIAZEPINE RECEPTORS

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The octapeptide cholecystokinin (CCK-8) and its more powerful analog cerulein have been shown, when administered systemically, to cause effects similar to those of tranquilizers of the benzodiazepine series [6, 8]. Relatively high doses of cerulein and CCK-8 prolong the latent periods of manifestation of seizures induced by thiosemicarbazide and harman, and also increase the threshold dose of picrotoxin required to induce seizures in mice [6, 8]; cerulein, moreover, has a stronger anticonvulsant action than diazepam. A similar anticonvulsant action also is observed in response to intraventricular injection of low doses of CCK-8 (1 and 100 ng) [4]. Meanwhile CCK-8 and cerulein have an action which differs significantly from that of tranquilizers of the benzodiazepine series. Cerulein, unlike diazepam, potentiates seizures induced by the GABA receptor antagonist bicuculline [6]. Injection of Ro 15-1788, a benzodiazepine antagonist, did not abolish the sedative and anticonvulsant action of cerulein and CCK-8 [8].

The aim of this investigation was to study the role of benzodiazepine receptors in the anticonvulsant action of cerulein.

## EXPERIMENTAL METHOD

Experiments were carried out on male mice weighing 25-30 g. When picrotoxin seizures were investigated cerulein (From Farmitalia — Carlo Erba, Italy) was injected in various doses (20-500 μg/kg) subcutaneously 10 min before intraperitoneal injection of picrotoxin in a dose of 8 mg/kg (from Serva, West Germany). In the experiments of series I the effect of cerulein (5-1000 nM) of binding of $^3$H-flunitrazepam (1 nM) in medium consisting of 50 mM Tris-HCl buffer solution, was determined. In the experiments of series II 120 mM KCl was added to this medium. There were 16-20 animals in each series. Three different parameters of picrotoxin seizures were determined: latent period of clonic convulsions, latent period of tonic convulsions, and length of survival of the mice after injection of 6 mg/kg of picrotoxin. The response of the mice to injection of picrotoxin was observed for 30 min. If in the course of that time the animals did not develop seizures or did not die, the response of the mice with respect to all parameters tested corresponded to 30 min. The CCK-8 antagonist proglumide (from Rotta Farmaceutici, Italy) [3] was injected intraperitoneally in doses of 5 and 25 mg/kg 5 min before injection of cerulein. Parallel with the study of the behavioral reactions, the effect of cerulein on binding of $^3$H-flunitrazepam was investigated in vitro and in vivo. Proglumide (5 and 25 mg/kg) and cerulein (20-500 μg/kg) were injected 5 min before subcutaneous injection of $^3$H-flunitrazepam in a dose of 0.3 μg/kg (specific activity 84 Ci/mmole, from Amersham Corporation, England). The animals (6 mice from each series) were decapitated 30 min after injection of the isotope. The forebrains of animals of the same group were pooled and homogenized in a Potter's homogenizer in 40 volumes of Tris-HCl buffer (50 mM, pH 7.4) at 20°C. Specific binding of flunitrazepam was determined after addition of 10 μM unlabeled flunitrazepam to the brain homogenates. The difference between the parameters of radioactivity of samples without ligand and with unlabeled ligand characterized specific binding of flunitrazepam. The samples were incubated at 0°C for 60 min. After incubation they were filtered through GF/B filters (Whatman, England), which were then washed twice with 5 ml of buffer. Radioactivity of the filters was determined in Bray's scintillator on an LS-7500 beta-particle counter (from Beckman, USA). Experiments to study binding of $^3$H-flunitrazepam in vitro in the mouse forebrain were carried out by the method described previously [1].

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TABLE 1. Effect of Cerulein and Proglumide on Picrotoxin Seizures and Binding of $^3$H-flunitrazepam *in Vivo* (M ± m, n = 3)

| Preparations | Dose | Specific binding of $^3$H-flunitrazepam in forebrain, counts/g tissue | Latent periods of picrotoxin seizures | | |
|---|---|---|---|---|---|
| | | | Clonic, sec | Tonic, min | Duration of survival of mice, min |
| Physiological saline | — | 14 970±829 | 417±23 | 13,4±1,4 | 13,8±1,4 |
| Cerulein | 20 | 14 840±850 | 428±36 | 13,5±1,8 | 14,0±1,7 |
| | 50 | 14 020±790 | 486±42 | 15,6±1,5 | 16,2±1,8 |
| | 100 | 12 200±680 | 593±41* | 19,3±2,3* | 21,3±2,5* |
| | 250 | 6 145±420*** | 674±58** | 19,8±1,5** | 21,6±2,0* |
| | 500 | 5 720±380*** | 573±62* | 20,4±2,7* | 21,0±2,8* |
| Proglumide | 5 | 15 030±790 | 432±32 | 13,6±1,5 | 14,2±1,5 |
| | 25 | 15 840±760 | 406±25 | 12,8±1,7 | 13,1±1,8 |
| Proglumide + cerulein | 5+100 | 10 820±860** | 644±48** | 20,8±1,5** | 23,4±1,9** |
| Proglumide + cerulein | 25+100 | 15 620±670 | 504±36 | 17,4±1,9 | 18,6±2,0 |
| Proglumide + cerulein | 25+250 | 11 640±870* | — | — | — |

Legend. *P < 0.05, **P < 0.01, ***P < 0.001.

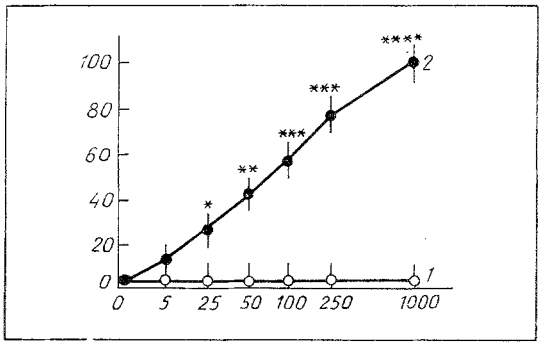


Fig. 1. Effect of cerulein (5-1000 nM) on binding of $^3$H-flunitrazepam (1 nM) *in vitro*. Abscissa, cerulein concentration (in nM); ordinate, percentage inhibition of binding of $^3$H-flunitrazepam by cerulein. Inhibitory action of 1 μM unlabeled flunitrazepam taken as 100%. 1) action of cerulein in medium of 50 mM Tris-HCl, 2) in medium of 5 mM KCl and 120 mM NaCl in 50 mM Tris-HCl. *P < 0.05 ** P < 0.01, ***P < 0.001, ****P < 0.0001 (by Student's t test). Results of three independent experiments are shown.

## EXPERIMENTAL RESULTS

Preliminary subcutaneous injection of relatively high doses of cerulein (over 100 μg/kg) delayed the development of picrotoxin seizures (Table 1); the latent period of clonic and tonic convulsions and the survival of the mice were lengthened. Cerulein in a dose of 250 μg/kg had the strongest effect on picrotoxin seizures, and a further increase in the dose of cerulein was not followed by stronger anticonvulsant action. In doses inhibiting picrotoxin seizures, cerulein significantly inhibited binding of $^3$H-flunitrazepam *in vitro*. In doses of 250 and 500 μg/kg cerulein caused a more than 50% decrease in specific binding of flunitrazepam in the forebrain. The CCK-8 antagonist proglumide, in the doses tested, caused no significant change in the picrotoxin seizures, and only in a dose of 25 μg/kg was there a very small increase, not significant, in specific binding of $^3$H-flunitrazepam (Table 1). In a dose of 5 mg/kg proglumide potentiated the inhibitory effect of cerulein both on pictrotoxin seizures and on specific binding of flunitrazepam. In a dose of 25 mg/kg proglumide had the opposite action: it reduced the antipicrotoxin effect of 100 μg/kg of cerulein (250 μg/kg) on specific binding of flunitrazepam. In experments *in vitro* cerulein (5-1000 nM) concentrated

binding of $^3$H-flunitrazepam only in the presence of 5 mM KCl and 120 mM NaCl (Fig. 1). Under these conditions, parallel with the increase in the concentration of cerulein, it was observed to have an inhibitory effect on binding of labeled flunitrazepam.

The results of this investigation demostrate the modulating effect of cerulein on benzodiazepine receptors. Starting with a dose of 100 μg/kg cerulein inhibited both picrotoxin seizures and specific binding of $^3$H-flunitrazepam in the mouse forebrain *in vivo*. These results show that the inhibitory effect of cerulein on picrotoxin seizures and on binding of $^3$H-flunitrazepam are realized through the same mechanisms. Involvement of cholecystokinin receptors in these effects of cerulein is demostrated by potentiation of the latter by a low dose (5 mg/kg) of proglumide, and also by their weakening after injection of higher dose (25 μg/kg) of proglumide. We know that low doses of cerulein (27 μg/kg) definitely prolong hexobarbital sleep [8]. In the current view the action of picrotoxin and barbiturates on animal behavior is realized through their direct effect on the chloride channel [2, 5]. In experiments in vitro cerulein inhibited binding of $^3$H-flunitrazepam only in the presence of significant concentrations of chloride anion, further evidence in support of interactions between cerulein and the chloride ionophore. On this basis it can postulated that it is through the chloride ionophore that CCK-8 and cerulein realized their anticonvulsant action, and that cerulein exerts its modulating effect on binding of $^3$H-flunitrazepam.

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