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Benzodiazepines and Their Antagonists Interfere With Opioid-Dependent Stress-Induced Analgesia

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ROVATI, L. C., P. SACERDOTE, P. FUMAGALLI, M. BIANCHI, P. MANTEGAZZA AND A. E. PANERAI. *Benzodiazepines and their antagonists interfere with opioid-dependent stress-induced analgesia.* PHARMACOL BIOCHEM BEHAV **36**(1) 123–126, 1990.—Timing or intensity of shocks significantly modify the characteristics of the analgesia induced by footshock, and conditioning to footshock induces analgesia, independently from the time and shock parameters used for conditioning. However, whatever the parameters of shock, and the presence of conditioning or not, the stress has to be inescapable in order to produce an increase in pain thresholds. This observation suggests that anxiety plays a major role in the development of stress-induced analgesia. In order to test this hypothesis we investigated the effects of the benzodiazepine agonists diazepam and clonazepam, the antagonists RO 15-1788, CGS 8216, CGS 9896, and the inverse agonists FG 7142 and FG 7041 on the development and maintenance of stress-induced analgesia. Benzodiazepine receptor agonists decreased the analgesic effect of inescapable footshock, benzodiazepine receptor antagonists increased the footshock induced analgesia, whereas inverse agonists did not modify the analgesia induced by the shock. All the benzodiazepine receptor ligands blocked the antagonism of the footshock analgesia induced by naloxone.

Stress Analgesia Footshock Benzodiazepines Anxiety

FOOTSHOCK stress increases pain thresholds (16), and timing or intensity of shocks significantly modify the characteristics of the analgesia induced (16). Keeping constant the intensity of shock at 2.5 mA, intermittent prolonged footshock (1 second every 5 seconds over twenty minutes) yields a naloxone-reversible analgesia, while a continuous (3 minutes) footshock induces an analgesia not reversible by naloxone (10,16). If the shock duration is fixed, and the intensity increased, analgesia shifts from naloxone-reversible to naloxone-nonreversible (15). However, footshock-induced conditioned-analgesia is always reversible by naloxone, independently from parameters of the shock used for conditioning (16). Finally, whatever the parameters of shock, and the presence of conditioning or not, the stress has to be inescapable in order to produce an increase in pain thresholds (16). The latter observation suggests that anxiety might play a major role in the development of stress-induced analgesia. If this is the case, benzodiazepines receptor agonists should decrease, and inverse agonists, being anxiogenic (12), increase the analgesia induced by stress. In order to test this hypothesis we investigated the effects of the benzodiazepine agonists diazepam and clonazepam, the antagonists RO 15-1788, CGS 8216, CGS 9896, and the inverse agonists FG 7142 and FG 7041 (2) on the development and maintenance of the naloxone-reversible stress-induced analgesia.

METHOD

Male Sprague-Dawley CD rats (Charles River, Calco, Italy) were housed at 22 ± 2°C with a normal dark:light cycle of 10:14 hours, for at least one week before experiments. All experiments were conducted in the morning beginning between 0900–1000 hr, and the analgesic thresholds were always measured by the same operator who ignored treatments. Footshock (2.5 mA, 50 Hz) was administered for 1 second every 5 seconds over twenty minutes [Panerai *et al.* (10)], yielding a classical naloxone-reversible analgesia. Pain thresholds were evaluated by the tail flick test before drug administration, before shock, immediately after the end of shock and 3, 5, 10, 15, 20 minutes thereafter. Changes in pain thresholds were expressed as percentage of the Maximal Possible Effect (% MPE). Maximal Possible Effect expresses the equation $[(TL-BL)/(ML-BL)] \times 100$, where BL is the mean basal latency measured before the first treatment was applied (normal values 3.5–4.0 sec); TL is the test latency measured after treatments and shock when this was applied; ML is the maximal latency accepted (8 sec), chosen in order to avoid tissue damage to the rat tail. All drugs were administered intraperitoneally. Benzodiazepine receptor ligands were administered 30 minutes before footshock, while naloxone was administered 30 minutes before footshock and immediately after the end of shock, according to

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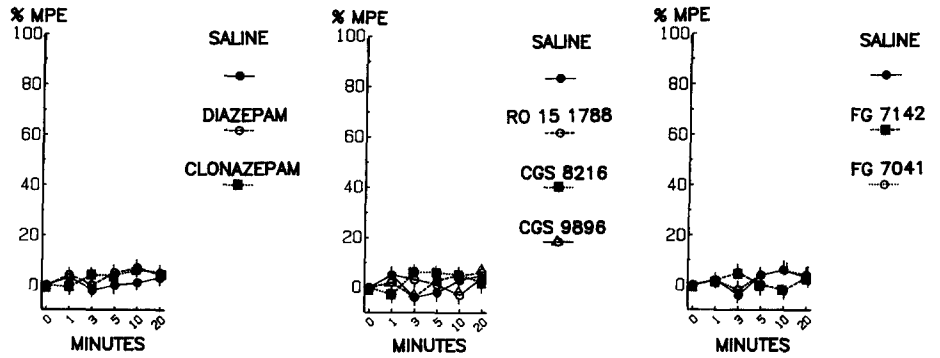

FIG. 1. Lack of analgesic effect of all benzodiazepine ligands used in this study.

previously tested schedules (10,16). In the first experiment, the drugs were tested for their potential analgesic effect. In the second experiment, the same drugs were tested on the analgesia elicited by footshock. Diazepam was used at the dose of 2.0 mg/kg, clonazepam at the dose of 1.0 mg/kg, and all other drugs the dose of 10 mg/kg. A similar dosing schedule was used when naloxone (5.0 mg/kg) was administered in order to antagonize stress-induced analgesia. In the binding studies, rats administered stress alone, stress and naloxone or stress and pretreated with diazepam (2.0 mg/kg) or RO 15-1788 (10.0 mg/kg) with or without naloxone (5.0 mg/kg), were decapitated immediately after stress, midbrain dissected out and placed in cold phosphosaline buffer (PBS), pH 7.4. Tissues were placed in 9.0 ml/g cold 0.01 M Tris buffer containing 0.32 M sucrose (final pH 7.5), homogenized, centrifuged twice at 43,500 × g for ten minutes twice, and the pellet frozen. Increasing concentrations (from 0.5 to 32 nM) of ^{3}H-naloxone (Amersham, specific activity 58.8 Ci/mmole) in the absence or presence of 10^{-6} M naloxone were incubated 30 minutes with 0.7 mg membrane protein at 25°C, and separated on GF-B Whatman filters. Specific binding ranged between 80% to 90% of total binding. The data presented represent the mean of three experiments.

Statistical evaluation of results was obtained by analysis of variance; in the binding studies, B_{max} and K_d were calculated by Scatchard plot analysis of binding data, and significances evaluated by Tukey's test for multiple comparisons.

RESULTS

Figure 1 shows that neither diazepam (2.0 mg/kg), clonazepam (1.0 mg/kg), RO 15-1788, CGS 8216, CGS 9896, FG 7142, nor FG 7041 at the dose of 10.0 mg/kg, i.e., the doses used in the experiments that follow, modified pain thresholds.

Both benzodiazepine receptor agonists decreased the analgesic effect of inescapable footshock ($p<0.01$), while benzodiazepine receptor antagonists RO 15-1788, CGS 8216, and CGS 9896 increased the footshock-induced analgesia ($p<0.01$), as it is shown in Fig. 2. The same figure also shows that the two inverse agonists FG 7142 and FG 7041 did not modify the analgesia induced by the shock. Figure 3 shows that all the benzodiazepine receptor ligands did interfere with the naloxone reversal of footshock-induced analgesia. It appears in fact that diazepam ($p<0.01$), clonazepam ($p<0.01$), RO 15-1788 ($p<0.01$), CGS 8216 ($p<0.01$), CGS 9896 ($p<0.01$), FG 7142 ($p<0.01$), and FG 7041 ($p<0.01$) inhibit the antagonism by naloxone.

Table 1 shows that shock induces a decrease of the number of binding sites that is prevented by pretreatment with naloxone. Treatment with diazepam or RO 15-1788 partially prevented the decrease in the number of opiate receptors induced by stress, and blocked the normalizing effect of naloxone on the decrease of receptors that follows stress.

DISCUSSION

The observation that diazepam and clonazepam, two benzodiazepine receptor agonists, decrease stress-induced analgesia is consistent with the hypothesis that anxiety might play a role in this response. On the contrary, the lack of effect of inverse agonists, that are by themselves anxiogenic (12), is not consistent with this hypothesis. Finally, it is surprising that benzodiazepine receptor antagonists, that are themselves deprived of any activity on the receptor (2), enhance the analgesia induced by footshock. The

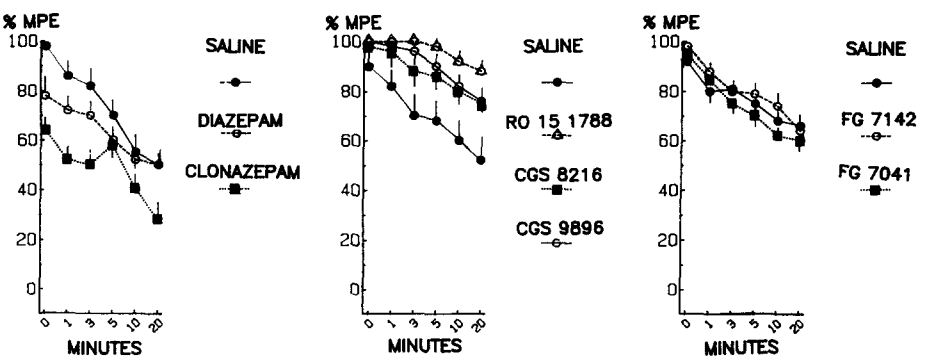

FIG. 2. Effect of the agonists (left panel), antagonists (middle panel), and inverse agonists (right panel) on the analgesia induced by footshock.

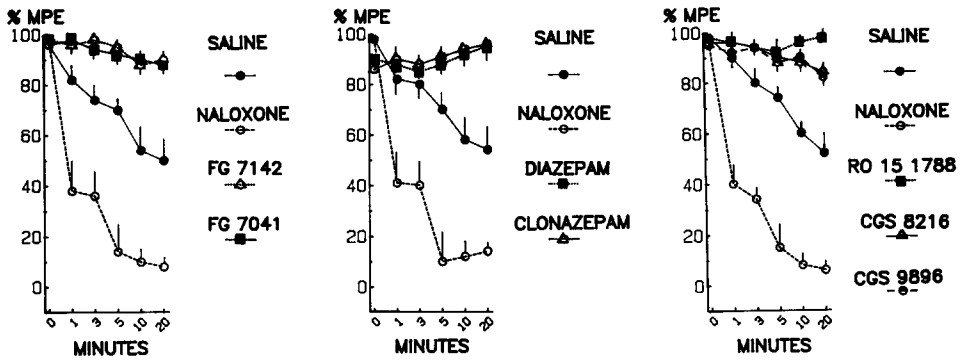

FIG. 3. Effect of the inverse agonists (left panel), agonists (middle panel), and antagonists (right panel) on the reversal of analgesia induced by naloxone.

TABLE 1

^{3}H-NALOXONE BINDING IN MIDBRAIN: EFFECT OF STRESS AND PHARMACOLOGICAL TREATMENTS

Experimental Group	B_{max} (10^{-12})
Controls	368.7 ± 13.8*
Stress	143.5 ± 19.3[a,c]
Stress + Naloxone	422.7 ± 24.0[b]
Stress + Diazepam	274.0 ± 22.1[a,b,c]
Stress + Diazepam + Naloxone	245.9 ± 9.0[a,b,c]
Stress + RO 15-1788	248.0 ± 19.0[a,b,c]
Stress + RO 15-1788 + Naloxone	124.2 ± 12.0[a,b,c]

*Mean ± S.D.
[a]$p<0.01$ vs. controls.
[b]$p<0.01$ vs. stress.
[c]$p<0.01$ vs. stress + naloxone.

whole of these observations is difficult to explain and leads to speculative considerations, e.g., anxiety might be not important for the development of stress-induced analgesia, or the drugs used might interfere with an endogenous benzodiazepine receptor agonist. Consistently with the latter hypothesis, Sangameswaran (14) showed the existence of an endogenous agonist for benzodiazepine receptor, and its activation by stress is predictable. In presence of an endogenous agonist, exogenous agonists such as diazepam and clonazepam might block the development of analgesia induced by footshock, being synergic to the endogenous agonist. On the contrary, inverse agonists might counteract the effect of the endogenous agonist and lead to no detectable effect. Consistently with this hypothesis, the antagonist RO 15-1788 might amplify the analgesia induced by footshock antagonizing the binding of the endogenous ligand on the receptor.

Also, the effect of benzodiazepine ligands on the reversal of analgesia induced by naloxone is difficult to explain and offers the side to speculation. Benzodiazepine ligands might activate a nonnaloxone-reversible analgesic pathway. Consistently with this hypothesis, all the benzodiazepine receptor ligands we used block adenosine reuptake (7,11), thus potentiate adenosine that is known to elicit a naloxone-nonreversible analgesia (1). At present, however, there are no experimental data to suggest this explanation of our results. The binding study shows that stress induces a decrease of opiate binding sites, and this decrease is prevented by naloxone, but not when the opiate receptor antagonist is administered to rats pretreated with diazepam or RO 15-1788. While the decrease of binding sites in stressed rats was previously explained with the possible occupancy of receptors by an endogenous agonist activated by stress (4), the effect of benzodiazepine receptor ligands cannot be explained with a direct effect on the receptor, since none of them binds the opiate receptor. Consistently with this observation, it was shown that both benzodiazepine receptor agonists and antagonists modulate the effects of endogenous and exogenous opiates through the GABAergic system, rather than directly at the opiate receptor (8,9).

Interestingly, benzodiazepine agonists and antagonists inhibit the increase of the binding of ^{3}H-naloxone present in the midbrain from stressed rats pretreated with naloxone and the effect of naloxone on the analgesia induced by footshock. Moreover, a careful survey of the data presented unveils that not only the antagonistic effect of naloxone is blocked by benzodiazepine receptor ligands, but an analgesic effect of naloxone shows up in our experimental conditions, similarly to that previously observed (13).

The lack of effect of benzodiazepine receptor antagonist that we observe on analgesic thresholds is in contrast with the analgesic effect of RO 15 1788 reported by Morgan *et al.* (6). The difference of results can be explained by the difference in doses. At 20 mg/kg RO 15 1788 begins to show agonistic properties that are fully evident at 50 mg/kg (5), the dose employed by Morgan (6). In this context, the analgesic effect of RO 15-1788 is consistent with the analgesic effect of benzodiazepine agonists such as diazepam, clonazepam, and chlordiazepoxide previously reported (3).

In conclusion, our data suggest that anxiety does not play a pivotal role in stress-induced analgesia, and that, as previously shown, stress can induce changes in the opiate receptor leading to changes in its specificity, as shown by the paradoxical effect of naloxone.

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