# WITHDRAWAL SYNDROME INDUCED BY THE BENZODIAZEPINE RECEPTOR ANTAGONIST CGS 8216 AFTER CHRONIC DIAZEPAM TREATMENT IN RATS

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The high anxiolytic, hypnotic, and sedative potential of the benzodiazepine tranquilizers suggests that mental and physical dependence may develop on these substances if they are used for a long time. Clinical data on the frequency of withdrawal symptoms after administration of benzodiazepines are contradictory. According to some workers [8], a withdrawal syndrome is extremely rare after discontinuation of benzodiazepines. Other workers [7, 14], on the other hand, consider that a withdrawal syndrome due to long-term administration of benzodiazepine tranquilizers and their subsequent discontinuation is seen quite frequently, although as a rule it is seen in abortive forms, and in many cases is not diagnosed by clinicians. Experiments on animals and clinical observations have shown that repeated administration of benzodiazepines gives rise to increased tolerance to their sedative and anticonvulsant effects [1, 2, 4]. Under experimental conditions chronic administration of benzodiazepines in low doses does not give rise to characteristic features of withdrawal, but behavioral changes observed in animals after their discontinuation constitute what has been called the rebound syndrome, characterized by enhanced emotional reactivity, general apathy, and automatic disturbances [3]. Investigators who have used very high doses of diazepam (over 100 mg/kg daily) [10] or chlordiazipoxide (110-800 mg/kg daily) [13] for 4 weeks have obtained a behavioral syndrome in rats which could be characterized as withdrawal. Withdrawal has been successfully obtained in these same animals by administration of the benzodiazepine receptor antagonist Ro 15-1788 [11, 12]. It must be noted, however, that withdrawal induced by Ro 15-1788 was weaker than the spontaneous withdrawal syndrome [11], evidently because of the weak benzodiazepine-like activity of Ro 15-1788 [5, 9].

Data are given in this paper to show that another benzodiazepine receptor antagonist, the triazoloquinoline derivative CGS 8216 [9], induces a well-marked behavioral syndrome which can be characterized as withdrawal in rats even when receiving a relatively short course of diazepam in low doses. Withdrawal induced by CGS 8216 may serve as a convenient model with which to study the addictive action of benzodiazepine tranquilizers.

## EXPERIMENTAL METHOD

Experiments were carried out on male rats weighing 220-280 g. The animals were given diazepam (Seduxen, from Gideon Richter, Hungary) intraperitoneally in doses of 5 to 10 mg/kg once only, or once daily for 5-30 days. At various times after discontinuation of the drug the animals were placed in plexiglas boxes, designed for visual observation of their behavior, and behavioral changes were recorded for 60 min; CGS 8216 was then given (in doses of 1, 2, 5, and 5 mg/kg, intraperitoneally), and the animals' behavior was again recorded. The results were subjected to analysis of variance (ANOVAR) followed by statistical analysis by the Mann–Whitney U test and the Fisher F test.

## EXPERIMENTAL RESULTS

After the end of daily administration of diazepam for 30 days in a dose of 10 mg/kg administration of CGS 8216 in a dose of 5 mg/kg gave rise to a behavioral syndrome whose basic features are given in Table 1. The most characteristic of them were head shakings, episodes of myoclonic twitching of the forelimbs, chewing movements, contraction of the tail muscles,

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TABLE 1. Characteristics of Behavioral Syndrome Induced by CGS 8216 in Rats 1-15 Days after Discontinuation of Diazepam, after its Administration for 30 Days

| Behavioral feature | Frequency of occurrence of feature, % | | | | |
|---|---|---|---|---|---|
| | days | | | | |
| | 1-st | 2 nd | 5th | 10th | 15-th |
| Head shakings | 87** | 90** | 80* | 54* | 24 |
| Myoclonus of the forelimbs | 97** | 86** | 80** | 48* | 12 |
| Chewing movements | 80** | 90** | 87** | 42 | 22 |
| Stereotyped sniffing | 40 | 47 | 20 | 18 | 10 |
| Enhanced emotional response to an irrelevant object | 63* | 78* | 57 | 48 | 28 |
| Contraction of tail muscles | 90* | 90** | 80* | 60* | 14 |

Legend. *$p < 0.05$, **$p < 0.01$ compared with group of rats with spontaneous withdrawal of diazepam and group of rats receiving a long course of injections of physiological saline followed by injection of CGS 8216 (F-test). Number of experiments was 30.

TABLE 2. Dependence of Intensity of Withdrawal Syndrome on Duration of Administration of CGS 8216 (2.5 mg/kg, intraperitoneally, M ± m)

| Duration of diazepam administration, days | Number of head shakings | Number of myoclonic twitches |
|---|---|---|
| 1 | 2,0±1,0 | 2,4±1,0 |
| 10 | 8,0±1,7 | 4,0±1,5 |
| 20 | 7,0±2,7 | 15,3±4,3 |
| 30 | 10,8±2,4 | 16,2±3,5 |

Legend. Diazepam was injected in a dose of 5 mg/kg once a day. CGS 8216 (2.5 mg/kg) was injected 48 h after discontinuation of diazepam.

enhanced emotional response to external stimuli, and sniffing. Incidentally, the behavioral features listed in Table 1 were also found in animals with spontaneous withdrawal of diazepam, and also in the control rats after receiving CGS 8216. However, they were much weaker in the latter and they were observed in fewer of the animals (30-40%). Analysis of variance showed that features such as head shaking, myoclonus of the forelimbs, enhanced emotional reactivity, and contraction of the tail muscles were significantly higher ($p < 0.01$) in animals receiving diazepam followed by CGS 8216 than in rats with spontaneous withdrawal or in rats receiving CGS 8216 alone. All these behavioral changes developed quite quickly, in the course of 5-7 min, and then continued for 1-1.5 h after administration of CGS 8216. A withdrawal syndrome could be induced by administration of CGS 8216 for 10 days after discontinuation of diazepam, but its intensity gradually diminished, and on the 15th day it no longer differed from that in rats with spontaneous diazepam withdrawal (Table 1). Correlation analysis of the time course of these features showed that a strong correlation was present between them all ($r = 0.84$-$0.99$, $p < 0.01$). Under these circumstances head shakings and myoclonic twitches of the forelimbs were observed most frequently. They were assessed quantitatively and they were therefore chosen as the basic features of the withdrawal syndrome. As the results of subsequent experiments showed, the intensity of the withdrawal syndrome depends on the duration of administration of diazepam (Table 2) and on the dose of CGS 8216 (Table 3). Naloxone, an antagonist of opiate receptors, did not induce the features of withdrawal described above in animals under chronic diazepam treatment. Diazepam (10 and 20 mg/kg) significantly inhibited withdrawal induced by CGS 8216, evidence of the specificity of the syndrome.

The experimental results are thus evidence that the benzodiazepine receptor antagonist CGS 8216 gives rise to a syndrome in animals previously receiving diazepam which can be characterized as a withdrawal syndrome. The individual features of this syndrome, such as enhanced emotional reactivity and head shakings, resemble the withdrawal syndrome induced in animals receiving morphine by the opiate receptor antagonist naloxone. The intensity of these features depends on the duration of diazepam administration and the dose of CGS 8216. The dose of the antagonist necessary to induce withdrawal corresponds to that necessary to abolish the action of diazepam under acute experimental conditions [6].

TABLE 3. Intensity of Withdrawal Syndrome in Rats Receiving Diazepam in a Dose of 5 mg/kg (20 days) Depending on Dose of CGS 8216 Injected 48 h after Discontinuation (M ± m)

| Experimental conditions | Number of head shakings | | Number of myoclonic twitches | |
|---|---|---|---|---|
| | control | diazepam | control | diazepam |
| Physiological saline | 1,2±0,6 | 7,0±2,9 | 0 | 0,3±0,1 |
| CGS 8216, mg/kg | | | | |
| 1,0 | 1,9+0,6 | 11,5±3,1 | 0,3±0,2 | 0,7±0,4 |
| 2,5 | 4,3±2,3 | 15,3±4,3* | 1,7±0,9 | 7,3±2,0* |
| 5,0 | 2,7±0,9 | 15,6±4,1** | 6,0±2,4 | 18,8±3,0** |

Legend. *$p < 0.05$, **$p < 0.01$ (Mann–Whitney U-test) compared with control. Each group contained seven animals.

It may be concluded that the withdrawal syndrome described in this paper and induced by the benzodiazepine receptor antagonist CGS 8216 in rats after long-term administration of diazepam is a convenient model for the study of the addictive potential of tranquilizers.

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