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Discriminative Stimulus Effects of Drugs Acting at $GABA_A$ Receptors: Differential Profiles and Receptor Selectivity

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SANGER, D. J., G. GRIEBEL, Gh. PERRAULT, Y. CLAUSTRE AND H. SCHOEMAKER. *Discriminative stimulus effects of drugs acting at $GABA_A$ receptors: Differential profiles and receptor selectivity.* PHARMACOL BIOCHEM BEHAV **64**(2) 269–273, 1999.—The $GABA_A$ receptor complex contains a number of binding sites at which a variety of psychotropic drugs, including benzodiazepines, barbiturates, and some neurosteroids, act to potentiate or inhibit the effect of the transmitter. Many studies have reported that these drugs can produce discriminative stimulus actions, but the cueing effects of compounds acting at different sites to enhance the effects of GABA are not identical. The discriminative stimulus effects of benzodiazepines have been analyzed in detail, and there is also a great deal of information available on the effects of non-benzodiazepine compounds acting at BZ(ω) recognition sites, which form part of the $GABA_A$ receptor complex. Of particular interest are compounds with selectivity for the $BZ_1(\omega_1)$ receptor subtype including zolpidem, zaleplon, and Cl 218,872. $BZ_1(\omega_1)$-selective drugs substitute for the discriminative stimulus produced by chlordiazepoxide only partially and at sedative doses. This is consistent with the view that sedative effects of BZ(ω) receptor agonists are mediated by the $BZ_1(\omega_1)$ receptor subtype, whereas the discriminative stimulus produced by chlordiazepoxide may be produced by activity at the $BZ_2(\omega_2)$ subtype. Analysis of this hypothesis is complicated by the variety of levels of intrinsic activity shown by different drugs. © 1999 Elsevier Science Inc.

Drug discrimination Benzodiazepine receptors Chlordiazepoxide Zolpidem Rats

GABA (γ-aminobutyric acid) is probably the most important inhibitory neurotransmitter in the mammalian central nervous system, and acts through at least three receptor subtypes ($GABA_A$, $GABA_B$, and $GABA_C$ receptors). The $GABA_A$ receptor is perhaps of particular significance for psychopharmacology because it contains a variety of binding sites at which behavior-modifying drugs act to produce some or all of their effects (25). Among these drugs are benzodiazepines, barbiturates, ethanol, and some neurosteroids. These agents produce many pharmacological effects, including discriminative stimulus actions in experimental animals and humans. Drug discrimination is, therefore, an effective and important technique for studying the behavioral pharmacology of $GABA_A$ receptors.

The drugs acting through $GABA_A$ receptors whose discriminative stimulus effects have been investigated most thoroughly are those that bind to the so-called benzodiazepine (BZ) or ω receptors, and this article will focus on these agents. However, many other drugs acting at the $GABA_A$ receptor complex have been found to produce discriminable cues. These include ethanol, barbiturates, muscimol (7), the neurosteroid pregnanolone (28), sodium valproate (15), pentylenetetrazole (24), and clomethiazole (27). There have been found to be different levels of cross-substitution between compounds that potentiate GABAergic transmission by acting at different sites on $GABA_A$ receptors. In some cases, such as with barbiturates and benzodiazepines, discriminative stimulus properties overlap considerably. In other cases, however, such as between direct agonists at the transmitter site, like muscimol and THIP, and compounds acting at modulatory sites, cross-substitution is much less (12). Thus, as pointed out some years ago (19), the behavioral effects of compounds acting at different sites can be distinguished by drug discrimination as well as by other behavioral methods.

STIMULUS EFFECTS OF BENZODIAZEPINES AND RELATED DRUGS

Experimental animals including rats, pigeons, rhesus monkeys, and baboons readily learn to discriminate benzodiaz-

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epines from placebo. The stimulus effects of this group of drugs have been analyzed in great detail following early studies using chlordiazepoxide (3) and diazepam (9). In general, there is complete cross-substitution between different benzodiazepines, and the potencies of different drugs to substitute for diazepam were found to correlate highly with their in vitro affinities to bind to BZ receptors and with human therapeutic doses (31). Also consistent with a BZ receptor-mediated effect are the findings that stimulus properties are antagonized in a dose-related way by flumazenil and other BZ receptor antagonists (10,18).

Drugs with nonbenzodiazepine chemical structures, including zolpidem, abecarnil, zopiclone, suriclone, and alpidem, but which also potentiate the effects of GABA by acting at BZ (ω) receptors, also produce discriminative stimulus effects, as would be expected. In some cases (see below) these can be distinguished, at least partially, from those of benzodiazepines suggesting different mechanisms of action. There is also some evidence that the discriminative stimulus effects of all benzodiazepines are not identical, with lorazepam (2) and alprazolam (30) showing more selective profiles.

CHLORDIAZEPOXIDE CUE IN RATS

Rats were trained to discriminate a dose of 5 mg/kg of chlordiazepoxide from saline using a standard fixed-ratio (FR)10 food reinforcement schedule during daily 15 min sessions [see (20) for more details of the procedure]. The discrimination was rapidly learned, and the chlordiazepoxide ED_{50} doses for drug lever choice and decreases in rates of responding were approximately 2 and 12 mg/kg, respectively (values differed only slightly for a number of experiments carried out during a period of over 10 years). The distinction between doses giving rise to the stimulus and those producing sedative effects was also found with other benzodiazepines believed to be full agonists at BZ(ω) receptors, suggesting that the discriminative stimulus is not related to sedation (3). This point is further reinforced by the finding shown in Fig. 1 that compounds known to act as partial agonists produced dose-related substitution for chlordiazepoxide but, in most cases, had no effect on rates of responding indicating a lack of sedation or ataxia, effects that normally require a high level of intrinsic efficacy (8).

Receptor Selectivity

The first suggestion that subtypes of BZ(ω) receptors might exist was based on the observation that the nonbenzodiazepine, Cl 218,872, bound to BZ(ω) receptors but showed a somewhat different pharmacological profile from that of the benzodiazepines themselves (13,14). CL 218,872 was described as showing selectivity for a $BZ_1(\omega_1)$ receptor subtype, whereas benzodiazepines showed equal affinity for and activity at both $BZ_1(\omega_1)$ and $BZ_2(\omega_2)$ subtypes. Recent research in molecular pharmacology and molecular biology has been able to characterize the structure of $GABA_A$ receptors, which have a pentameric form containing different subunits called α, β, γ, etc. (25). The BZ(ω) binding site is now known to be associated with the α and γ subunits. The pharmacologically defined $BZ_1(\omega_1)$ receptor subtype seems to correspond to $GABA_A$ receptors containing α_1 subunits, whereas the $BZ_2(\omega_2)$ subtype is heterogeneous corresponding to $GABA_A$ receptors with α_2, α_3, or α_5 subunits (16,17).

$GABA_A$ receptors containing different α subunits do not show a homogenous distribution in the CNS, and it has been suggested that different receptor subtypes may have different functional roles (21). A number of compounds, including Cl 218,872, zolpidem, alpidem, abecarnil, and zaleplon, have been found to show $BZ_1(\omega_1)$ selectivity, corresponding to higher affinity for $GABA_A$ receptors containing α_1 subunits than those containing α_2, α_3, or α_5 subunits. In the case of zolpidem, there is no affinity for, or activity at, α_5 containing

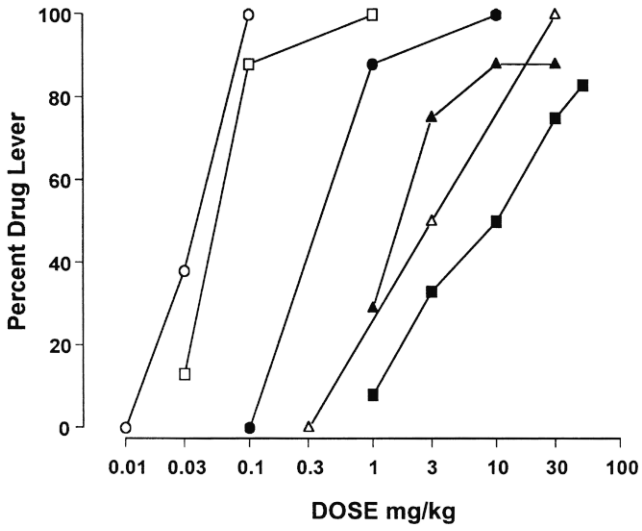

FIG. 1. Dose-related responding on the drug-associated lever produced by BZ(ω) receptor partial agonists in rats trained to discriminate chlordiazepoxide (5 mg/kg, IP) from saline. The drugs tested were: ○ imidazenil, ● Ro 17.1812, □ Bretazenil, ■ CGS 9896, △ ZK 91296, and ▲ Y23684. Results are shown as the percentage of animals tested choosing the chlordiazepoxide lever.

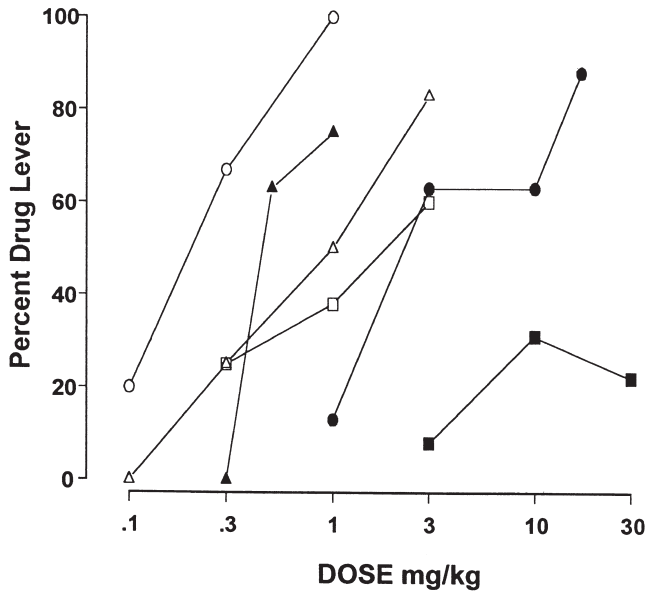

FIG. 2. Dose-related responding on the drug-associated lever produced by $BZ_1(\omega_1)$ selective compounds in rats trained to discriminate chlordiazepoxide (5 mg/kg, IP) from saline. The drugs tested were: ○ SX 3228, ● CL 218,872, □ zolpidem, ■ alpidem, △ zaleplon, and ▲ abecarnil. Results are shown as the percentage of animals tested choosing the chlordiazepoxide lever.

TABLE 1

POTENCIES OF BZ1 (ω1) SELECTIVE COMPOUNDS TO SUBSTITUTE FOR THE CHLORDIAZEPOXIDE (5 mg/kg) CUE AND TO DECREASE RATES OF LEVER PRESSING IN RATS

	ED_{50} mg/kg, IP	
Compound	Substitution	Response Rates
SX 3228	0.2	0.4
Abecarnil	0.6	0.3
Zaleplon	0.9	1.3
Zolpidem	1.9	1.4
CL 218,872	3.8	9.5
Alpidem	>30	30

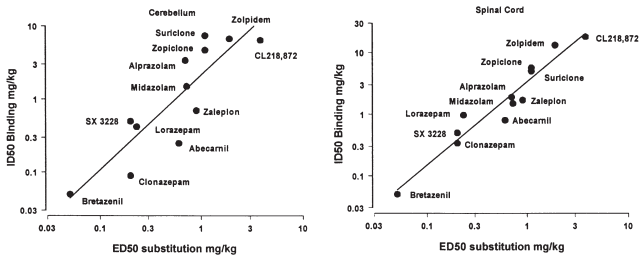

FIG. 3. Regression lines showing the relationship between the potencies of different compounds to substitute for the chlordiazepoxide cue (ED_{50} doses in mg/kg, IP) and to displace ^{3}H-flumazenil binding in vivo (ID_{50} doses mg/kg, IP). Data are shown for the cerebellum, which contains over 95% of the $BZ_1(\omega_1)$ receptor subtype and the spinal cord, which contains approximately 20% of the $BZ_1(\omega_1)$ receptor subtype.

receptors (5,11). These compounds have been used in drug discrimination experiments in rats, and while there was found to be some overlap with the stimulus effects of benzodiazepines and other nonselective compounds, this was far from complete (1,6,22,23,29). It seems likely, therefore, that different stimulus effects of drugs can be produced by actions at different $GABA_A$ receptor subtypes.

Figure 2 shows the effects of several compounds described as showing $BZ_1(\omega_1)$ selectivity in rats trained to discriminate chlordiazepoxide. With the exception of alpidem, all the drugs produced dose-related responding on the chlordiazepoxide-associated lever, but only with the highest dose of SX 3228 did all animals choose this lever. In fact, as is shown in Table 1, significant drug lever responding occurred only at doses that also greatly decreased response rates. This observation is consistent with the view that sedative/hypnotic activity of $BZ(\omega)$ receptor agonists is mediated by $BZ_1(\omega_1)$ receptor subtypes so that $BZ_1(\omega_1)$ selective compounds have particularly marked sedative effects (21). These results also seem consistent with the idea that the discriminative stimulus produced by chlordiazepoxide is probably not mediated by activity at $BZ_1(\omega_1)$ receptors. Of course, if this is the case, higher doses of $BZ_1(\omega_1)$ selective compounds would be expected to lose their receptor selectivity and give rise to a chlordiazepoxide-like stimulus. However, because animals do not respond at higher doses, it is not possible to test this hypothesis with the present method.

A corollary of the finding that low, nonsedative doses of $BZ_1(\omega_1)$ selective compounds do not produce a discriminative stimulus identical to that of nonselective drugs is that the chlordiazepoxide stimulus may be mediated by activity at $BZ_2(\omega_2)$ receptor subtypes. This hypothesis cannot be tested directly at the present time because no compounds with clear $BZ_2(\omega_2)$ selectivity are available for behavioral studies. However, some indirect evidence does exist. Sanger and Benavides (20) argued that, if the chlordiazepoxide cue were produced by activity at $BZ_2(\omega_2)$ sites, the potencies of different compounds to substitute for chlordiazepoxide should correlate more highly with the binding affinities of the same compounds in $BZ_2(\omega_2)$-rich areas of the CNS than in areas containing predominantly $BZ_1(\omega_1)$ sites. This prediction was confirmed when it was found that statistically significant correlations were found between behavior and in vivo displacement of ^{3}H-flumazenil in the spinal cord [80% $BZ_2(\omega_2)$], hippocampus [60% $BZ_2(\omega_2)$], and striatum [50% $BZ_2(\omega_2)$], but not in the cortex [35% $BZ(\omega_2)$] or cerebellum [< 5% $BZ_2(\omega_2)$].

Since this analysis was carried out, several novel compounds have become available that show selectivity for $BZ_1(\omega_1)$ sites, and it was considered of interest to carry out a similar analysis once again. (Although, clearly, the availability of $BZ_2(\omega_2)$ selective agents would provide a much better means of investigating this question). Therefore, the potencies to substitute for chlordiazepoxide shown by the nonselective agents bretazenil, clonazepam, lorazepam, midazolam, alprazolam, zopiclone, and suriclone and the $BZ_1(\omega_1)$ selective compounds abecarnil, SX 3228, zaleplon, zolpidem, and CL 218,872 were compared with potencies of the same drugs to displace flumazenil from the cerebellum or spinal cord. The resulting regression lines are shown in Fig. 3. There is clearly a very strong and significant correlation between drug-lever responding and binding in the $BZ_2(\omega_2)$-rich spinal cord (r = 0.96), but there was also a significant correlation for binding in the cerebellum (r = 0.73).

Although this result is perhaps consistent with the notion that $BZ_2(\omega_2)$ receptors are of relatively greater importance than $BZ_1(\omega_1)$ sites in mediating the discriminative stimulus produced by chlordiazepoxide, it clearly does not allow this conclusion to be drawn with any great confidence. There are, of course, a number of shortcomings of this correlational approach to look for mechanisms underlying behavioral effects of drugs. One is the assumption that all the compounds studied have roughly similar levels of intrinsic activity at the receptors under investigation. This is clearly not the case, as at least one of these compounds, bretazenil, is a partial agonist probably at all receptor subtypes.

Bretazenil would, thus, be expected to produce the chlordiazepoxide cue at doses that occupied a greater proportion of $BZ(\omega)$ sites that do nonselective compounds. This was investigated directly by calculating the proportion of $BZ(\omega)$ sites occupied in the cerebellum and spinal cord at doses that produced a 50% substitution for chlordiazepoxide. Figure 4 shows the results. As predicted, bretazenil occupied 50% of receptors in both CNS regions, a considerably higher percentage than that shown by many of the other drugs studied. High levels of receptor occupancy were also shown by clonazepam and abecarnil, consistent with the view that these compounds may also be partial agonists (8,26). At the other end of the scale, zolpidem, which in any event produced only partial substitution for chlordiazepoxide [Figure 2; (4)] at sedative doses, was active at doses occupying relatively low percentages of receptors in both regions. This would suggest that in vivo zolpidem has particularly high intrinsic activity, a result consistent

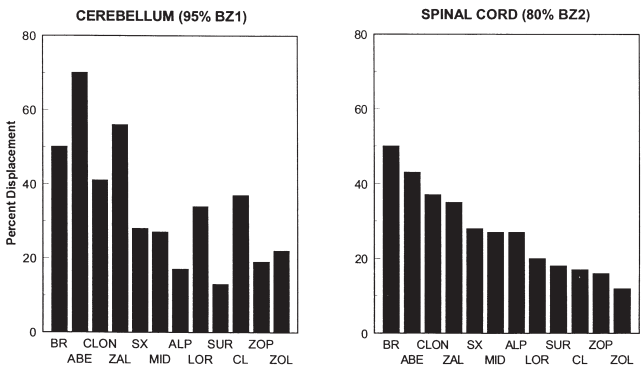

FIG. 4. Calculated values for the percentage displacement of ^{3}-flumazenil binding in the cerebellum and spinal cord of rats at ED_{50} doses to substitute for chlordiazepoxide. The drugs shown are bretazenil (BR), abecarnil (ABE), clonazepam (CLON), zaleplon (ZAL), SX 3228 (SX), midazolam (MID), alprazolam (ALP), lorazepam (LOR), suriclone (SUR), CL 218,872 (CL), zopiclone (ZOP), and zolpidem (ZOL).

with electrophysiological findings (11). On the other hand, results shown in Fig. 4 with some of the other compounds (e.g., zaleplon) do not seem to correspond completely with what is known of their pharmacological effects.

These results emphasize how hazardous it may be to use such correlations, even between behavior and in vivo biochemical results obtained under similar conditions, to draw conclusions about the functional roles of different receptor subtypes. It might also be noted here that this approach, involving data obtained from different regions of the rat CNS, provides no information on and makes no assumptions about the regions mediating the cueing effects of benzodiazepines or other drugs. Although it seems likely that different $GABA_A$ receptor subtypes are involved in the discriminative stimulus effects of different members of this class of drugs, a conclusion also drawn recently by Rowlett and Woolverton (18) on the basis of sophisticated pA_2 and pK_B analyses of data available in the literature, more selective compounds will probably be required before the function of particular receptor subtypes can be defined with greater precision.

CONCLUSIONS

Experimental animals can be trained to discriminate compounds acting at a variety of sites on the $GABA_A$ receptor complex. This method, therefore, has considerable potential for allowing a fine analysis of the pharmacology of drugs that potentiate or inhibit GABAergic neurotransmission. Although the discriminative stimulus effects of compounds acting at BZ(ω) binding sites have been investigated in some depth, a number of questions deserve further study. These include the significance of BZ(ω) receptor subtypes in mediating different stimulus effects. It can be argued that the $BZ_2(\omega_2)$ subtype is of particular importance in the cue produced by chlordiazepoxide. However, a direct test of this hypothesis will not be possible until compounds highly selective for $BZ_2(\omega_2)$ sites become available.

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