Pharmacology Biochemistry & Behavior, Vol. 38, pp. 769–773. © Pergamon Press plc, 1991. Printed in the U.S.A. 0091-3057/91 $3.00 + .00

Effects of Anxiety Drugs on the Modification of the Acoustic Startle Reflex by Noise Gaps

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Received 4 May 1990

HIJZEN, T. H., H. J. RIJNDERS AND J. L. SLANGEN. *Effects of anxiety drugs on the modification of the acoustic startle reflex by noise gaps.* PHARMACOL BIOCHEM BEHAV **38**(4) 769–773, 1991.—The acoustic startle reflex was modified by presenting gaps (0–30 ms) in a continuous noise (70 dB) before the startle eliciting stimulus. The effects of midazolam (0.4–1.8 mg/kg, IP), DMCM (0.1–0.4 mg/kg, IP), buspirone (5–20 mg/kg, PO), 8-OH-DPAT (0.5–8 mg/kg, IP), and clonidine (0.009–0.08 mg/kg, IP) on startle amplitudes (reflecting sensorimotor reactivity) on the one hand, and on gap inhibition of the startle reflex (a measure of temporal acuity) on the other hand, were investigated. The results showed that midazolam and clonidine attenuated sensorimotor reactivity dose-dependently. DMCM had no systematic effect on sensorimotor reactivity. Buspirone and 8-OH-DPAT increased sensorimotor reactivity dose dependently. Furthermore, midazolam and clonidine did not affect the neuronal systems underlying inhibition of the startle reflex. In contrast, DMCM and buspirone increased inhibition dose-dependently, and 8-OH-DPAT first increased and than decreased inhibition of the startle reflex. The possibility that drug-induced behavior affected the startle reflex was discussed.

Acoustic startle reflex Gap inhibition Sensorimotor reactivity Anxiety drugs Rats

GAP inhibition of the acoustic startle reflex in the rat can be observed when a steady background noise is interrupted during a few ms, at an appropriate lead time before the startle eliciting stimulus (17). Wecker and Ison (30) suggested that gap inhibition depends upon the temporal aspects of sensory stimulus processing ("temporal acuity"), and reported that gap inhibition of the startle reflex was attenuated after administration of alcohol, a drug which was supposed to "slow neural transmission along the primary auditory pathway beginning with the cochlear nucleus." This suggests that the gap-startle (G-S) paradigm may be used to study the effects of drugs on temporal acuity [cf. (18,32)]. However, drugs may also modify the startle reflex by an effect on the motor components of the primary startle pathway (4). Furthermore, several findings indicate that the neuronal systems underlying inhibition of the startle reflex, may be localized outside the primary startle pathway (22,23), and that the inhibition and the elicitation of startle are independent processes (11, 26, 33). Therefore, a modification of the startle reflex may also be produced by a drug effect on these neuronal systems. Apparently, drug effects on the sensory and the motor components of the startle pathway, and on inhibition of the startle reflex, are not directly separable. However, when drugs affect startle-alone (S) and G-S amplitudes equally, a common cause, i.e., a drug-induced change in the sensory and/or motor components of the primary startle pathway, may be assumed. In contrast, when a drug affects the neuronal mechanisms involved in inhibition of the startle reflex, the effects produced on S and G-S trials will be independent. Thus an analysis of covariance (ANCOVA), with the S amplitudes as covariates for the G-S amplitudes, can separate drug effects on sensorimotor reactivity from drug effects on inhibitory mechanisms.

The present study used the ANCOVA approach to investigate the effects of anxiety drugs on the inhibition of the startle reflex and on sensorimotor reactivity. Kellogg et al. (19) reported that diazepam decreased the effect of noise gaps on startle amplitudes. The first objective of the present study was to investigate whether the results obtained with diazepam are also found with midazolam, a more short-acting benzodiazepine (BDZ) agonist. Since BDZ inverse agonists and BDZ agonists generally affect behavior in opposite ways, it was also investigated whether DMCM (methyl-6,7-dimethoxy-4-ethyl-beta carboline-3-carboxylate), a potent inverse BDZ agonist with anxiogenic effects (16), enhanced gap effects on startle.

Anxiolytic effects have been reported with a variety of drugs, and these drugs may differ in their effects on basic functions. Therefore, the effects of buspirone, clonidine and 8-OH-DPAT [8-hydroxy-2-(di-n-propylamino) tetralin], three nonbenzodiazepine drugs with anxiolytic properties (3, 13, 14), on inhibition of the startle reflex and on sensorimotor reactivity were also investigated.

METHOD

Subjects

Sixty male rats of an outbred Wistar strain (CPB, Zeist, The

Netherlands) weighing 200–250 g at the beginning of the experiment, were housed four to a cage (60 × 36 × 20 cm). Subjects had free access to food and water. Room temperature was 20–22°C. The experiment was conducted during the second half of the non-reversed 12-h light-dark cycle (light: 7.00 a.m.–7.00 p.m.).

Apparatus

The startle device was a small rigid chamber (20 × 12 × 15 cm) attached to a heavy superstructure of nylatron and aluminum. The chamber was constructed of stainless steel rods with a nylatron top and connected to the superstructure by small fiberglass plates. A Bruel and Kjaer (Naerum, Denmark) accelerometer (type 4381) was attached to the top of the cage. The charge amplifier (type 2635) was switched in the velocity position. Startle stimuli (9 kHz; 116 dB, SPL; 20 ms) were presented through a Motorola piezo electric tweeter situated 12 cm from one side of the cage. The startle device was situated in a sound-attenuating room (Industrial Acoustics Co., New York) in which a noise of 70 dB (A) was provided by a random white noise generator (type 231R, Peekel, Rotterdam). Tone and noise intensities were measured inside the cage with a Bruel and Kjaer soundlevel meter type 2203. Tests were controlled by an Apple II-e computer with a GEN-65 function generator interface from Northwest Instruments, Beaverton, OR. Startle amplitudes were sampled each ms and the maximum value obtained within 200 ms after stimulus presentation was used for further data processing.

Procedure

One week before the first experimental session 20 startle-eliciting stimuli (116 dB) were presented to each animal. The results were used to subdivide the animals into 5 groups of 12 rats with similar mean startle amplitudes.

Each test session started with an adaptation period of 5 min followed by ten blocks of 5 startle trials. Startle trials differed in respect to noise-gap duration, i.e., background noise was interrupted during 0, 4, 7, 12 and 30 ms. Time between the beginning of the noise-gap and the startle stimulus was always 190 ms. Duration of noise gaps was randomized within each block of 5 trials. The intertrial interval was 20–30 s.

Data Reduction and Statistics

Startle amplitudes were averaged over the ten trials of the same type (gap duration). Trials in which noise was not interrupted before the startle stimulus was presented (startle-alone) were subjected to a classical multivariate two-way analysis of variance (MANOVA) with Drugs as a between factor having 5 levels and Doses as a within factor having 4 levels. A Doses × Drugs effect (if any) was analysed further by tests for linear, quadratic and cubic trends across doses, for each drug separately (20,25).

The data were also subjected to an analysis of covariance (ANCOVA). Linear, quadratic and cubic trend scores (across doses) were computed (20) for both startle-alone and gap data. Subsequently, the former were entered as covariates for the latter in the ANCOVA, which further included the within-factor Gaps and the between-factor Drugs. The ANCOVA was conducted by way of the program MULTIVARIANCE (12). Significant effects of drugs on doses trend scores were analysed further by testing trends for each drug separately, after adjustment for covariation with the equivalent startle-alone trend scores.

Drugs

Midazolam (0.45–0.9–1.8 mg/kg, IP, Hoffmann-La Roche, Switzerland) was dissolved in 0.9% saline and administered 10 min before the test. DMCM (0.1–0.2–0.4 mg/kg, IP, Schering, FRG) was dissolved in distilled water with a few drops of 0.15 N HCl and was administered 5 min before the test. Buspirone (5–10–20 mg/kg, PO, Bristol Myers, USA) was dissolved in 0.9% saline and administered 10 min before the test. 8-OH-DPAT (0.5–2.0–8.0 mg/kg, IP, Research Biochemicals, USA) was dissolved in 0.9% saline and administered 5 min before the test. Clonidine (0.0089–0.028–0.08 mg/kg, IP, Sigma, USA) was dissolved in 0.9% saline and administered 10 min before the test. Drugs were given in an end-volume of 0.6 ml/subject.

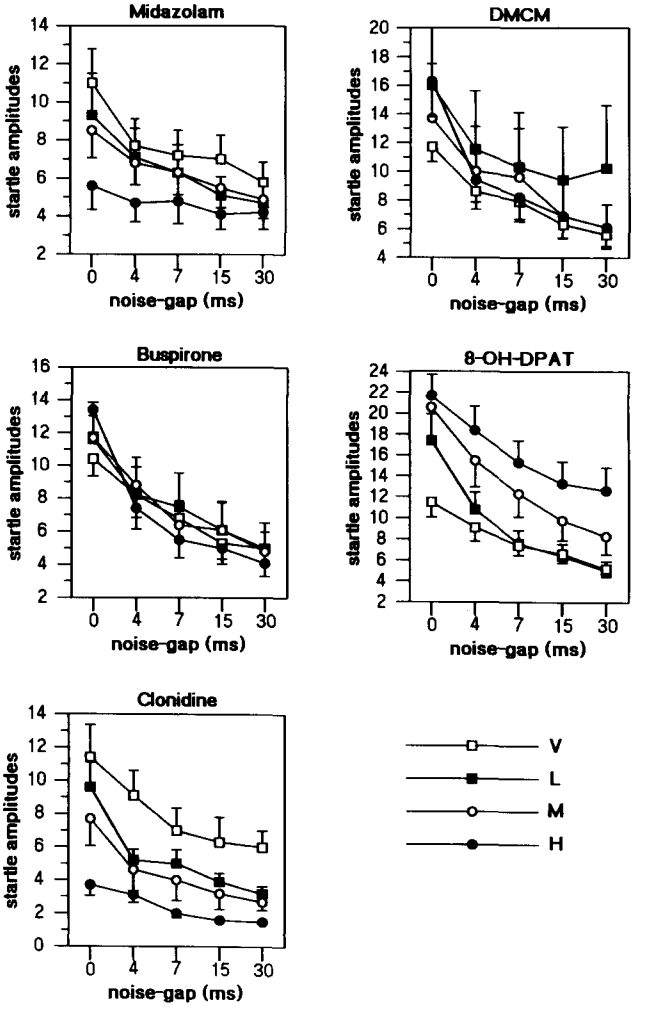

FIG. 1. Effects of drug doses on startle-alone and on gap-startle amplitudes. Doses: V(ehicle), L(ow), M(iddle), H(igh). Startle in arbitrary units. Vertical bars indicate the standard error of the mean.

RESULTS

The effects of drug doses on startle and on gap-inhibition of the startle reflex are presented in Fig. 1. Drugs had significantly different effects on startle-alone (S) amplitudes, $F(4,55) = 5.1$, $p<0.005$. The drugs × doses interaction was also significant, $F(12,140) = 4.8$, $p<0.001$. The analyses for linear trends showed that startle-alone amplitudes were dose-dependently attenuated by midazolam, $F(1,11) = 8.5$, $p<0.05$ and clonidine, $F(1,11) = 21.0$, $p<0.001$, and dose-dependently increased by buspirone,

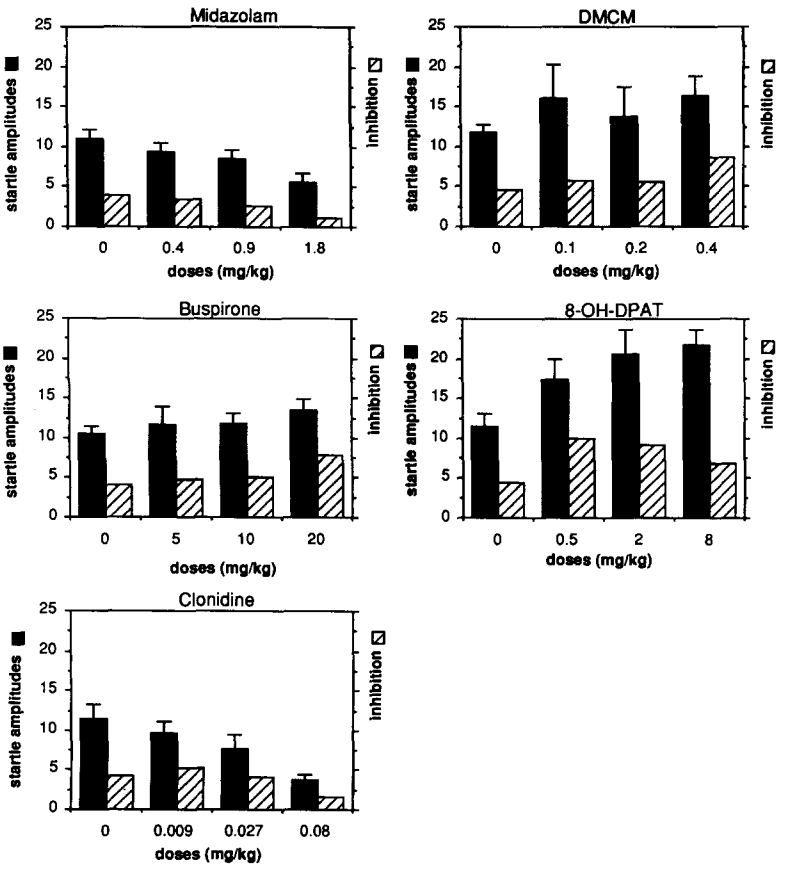

FIG. 2. Effects of drug doses on startle-alone amplitudes and on gap inhibition of the startle reflex. Inhibition is represented by the absolute differences between startle-alone amplitudes and the amplitudes produced when the startle eliciting stimulus was preceded by noise gaps. Vertical bars indicate the standard error of the mean. Startle in arbitrary units.

$F(1,11)=5.9$, $p<0.05$ and 8-OH-DPAT, $F(1,11)=25.3$, $p<0.001$. DMCM had no dose-dependent effect, although a post hoc t-test showed a significant difference between 0.4 mg/kg and vehicle amplitudes ($p<0.05$). None of the quadratic and cubic trends were significant.

According to the ANCOVA (MULTIVARIANCE), startle amplitudes decreased when the duration of the noise gaps was increased, $F(1,53)=3.5$, $p<0.005$. Attenuation of the startle amplitudes, produced by gaps of different durations did not depend on the drug dose administered, i.e., the drugs × dose × gaps interaction was not significant. Hence, further analyses were restricted to the effects of drug doses on startle amplitudes averaged over different gap durations. Figure 2 summarizes the S amplitudes and G-S amplitudes, averaged over gap durations.

The ANCOVA, with the linear, quadratic and cubic trends present in the startle-alone data as covariates for the trends in the gap-startle data resulted in a linear, $F(4,54)=6.1$, $p<0.001$, and a quadratic, $F(4,54)=7.4$, $p<0.001$, drugs times doses effect. The significant main effects were further analysed by an ANCOVA for each drug separately. According to these analyses neither midazolam nor clonidine affected the inhibition of the startle reflex. DMCM, $F(1,11)=5.5$, $p<0.05$, and buspirone, $F(1,11)=12.3$, $p<0.005$, increased inhibition linearly. The quadratic trend over doses was significantly for 8-OH-DPAT only, $F(1,11)=15.7$, $p<0.005$, i.e., 8-OH-DPAT first increased and then decreased inhibitory processes.

DISCUSSION

Startle-alone (S) amplitudes were dose dependently attenuated by midazolam and clonidine. The effect of DMCM on startle did not vary systematically with dose. Buspirone and 8-OH-DPAT increased S-amplitudes dose-dependently. Most of these effects were reported previously (6, 7, 9, 10, 16, 29). Drug effects on the S-amplitudes reflect a change in sensorimotor reactivity. Drug effects on Gap-Startle amplitudes reflect a change in sensorimotor reactivity and/or a change in the activity of the neuronal systems underlying inhibition of the startle reflex. In this study, drug effects on sensorimotor reactivity and on the inhibition of the startle reflex were separated by way of an ANCOVA.

According to the ANCOVA, midazolam had no effect on inhibition of the startle reflex, i.e., the decrease in startle amplitudes after noise gaps present in Fig. 2 is fully explained by the dose-dependent linear trend in startle-alone data. Berg and Davis (2) reported that depressant effects of benzodiazepines are not produced when startle is elicited electrically from the ventral cochlear nucleus, indicating that benzodiazepines depress acoustic startle very early in the acoustic startle pathway. Therefore, the present results are best explained as an effect of midazolam on early stimulus processing. This result is in agreement with the

diazepam-induced deficit in stimulus detection reported by Kellogg et al. (19).

Although 0.4 mg/kg DMCM increased startle amplitudes above vehicle values, there was no systematic effect of doses. Moreover, animals reacted in a highly individual way at the various doses (Figs. 1 and 2). Therefore, a more definite interpretation of the effect of DMCM on sensorimotor reactivity and gap inhibition should await further study.

The effects of clonidine, an alpha-2 receptor agonist with anxiolytic properties, are qualitatively similar to the effects of midazolam. Both drugs had no effect on the inhibition of startle, and decreased sensorimotor reactivity. Davis et al. (8) elicited startle responses electrically from different points along the startle pathway after systemic administration of clonidine and concluded that clonidine alters startle by decreasing neural transmission at sites within the startle circuit in or prior to the nucleus pontis caudalis as well as by actions in the spinal cord. Therefore, while midazolam appears to affect the sensory components of the startle pathway, the present effects of clonidine may be best explained by a predominant effect on the motor components of the startle pathway.

Buspirone and 8-OH-DPAT increased sensorimotor reactivity. Moreover, buspirone increased the inhibitory effect of noise gaps significantly and 8-OH-DPAT first increased and then decreased inhibition. As discussed in the introduction, these results suggest that buspirone and 8-OH-DPAT, in contrast to midazolam and clonidine, may have affected temporal acuity, i.e., temporal aspects of sensory stimulus processing.

8-OH-DPAT is known as a selective 5-HT_{1a} agonist (24). At relatively low doses 8-OH-DPAT binds to the presynaptic (auto)receptors of the raphe nuclei, higher doses may affect 5-HT postsynaptic receptors (15). The curvelinear relationship between doses and gap inhibition (Fig. 2) suggests, therefore, that gap inhibition depends upon presynaptic 5-HT_{1a} activity. Because buspirone has a substantial affinity for the 5-HT_{1a} receptor (27), the enhanced inhibition found in buspirone treated animals may be explained in a similar way. Further research is needed to substantiate this proposition.

It is also possible that the drugs evoked behavioral patterns which subsequently affected the startle response. In several studies, lower startle amplitudes were found during periods of spontaneous activity compared to periods in which the animals were quiet (21,31). Also, less prepulse inhibition was found during active periods (31). Although animal activity could not be observed in the present setting, signs of the 5-HT syndrome were generally observed before and after the test session when 8 mg/kg 8-OH-DPAT was administered. Other studies with 8-OH-DPAT reported a full-blown 5-HT syndrome at high doses (28). Obviously, the increase in 8-OH-DPAT-induced stereotyped behavior did not attenuate startle amplitudes after noise gaps. A similar finding was reported by Davis (5), i.e., d-amphetamine-induced stereotyped behavior did not attenuate prepulse inhibition. Futhermore, behavioral activity depressed startle-alone and prepulse-startle amplitudes (31). In the present study, 8 mg/kg 8-OH-DPAT had opposite effects on startle-alone and G-S amplitudes. Apparently, a negative correlation between activity and startle is not found in all experimental settings. Furthermore, Barbeau and Rossignol (1) reported that drugs may have qualitatively different effects on locomotor patterns. Taken together, these findings suggest that drugs may affect the startle response via an effect on behavioral activity and/or locomotor patterns.

In conclusion, midazolam and clonidine had no effect on gap inhibition of the startle reflex, a possible index of temporal acuity. The DMCM results were difficult to interpret because of the great variability within the data, and the absence of a dose-dependent effect. Buspirone and 8-OH-DPAT increased sensorimotor reactivity. In addition, buspirone increased and 8-OH-DPAT first increased and than decreased gap inhibition of the startle reflex. The involvement of 5-HT_{1a} receptors in the neuronal mechanisms underlying gap inhibition were discussed.

The possibility that the effects on startle-alone and on gap-startle amplitudes are influenced by drug-induced behavior was also discussed.

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