Footshock-induced freezing behavior in rats as a model for assessing anxiolytics

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Abstract. A number of chemically distinct anxiolytics were examined for effects on defensive behavior (footshock-induced freezing) in rats. Central nervous system acting drugs which are not anxiolytics were also studied. Animals were injected with a drug or vehicle (IP) prior to being placed in a chamber with a grid floor through which two footshocks were delivered. Behavior was observed during the pre-shock period (2 min) and for 4 min after the second footshock. The effects of the following drugs on the duration of footshock-induced freezing were studied: diazepam (DZP); 2-amino-4,5-(1,2-cyclohexyl)-7 phosphonoheptonic acid (NPC 12626); 3-((+/-)-2-carboxypiperazine-4-yl)-propyl-1-phosphonic acid (CPP); [(+)-5-methyl-10-11,dihydroxy-5H-dibenz(a,d)cyclohepten-5,10-imine (MK-801); buspirone hydrochloride (BUS); dl-amphetamine sulfate (AMP); haloperidol (HAL); ethyl-β-carboline-3 carboxylate (β-CCE). Compounds which reduced the duration of footshock-induced freezing included DZP, BUS, and the competitive NMDA antagonists NPC 12626 and CPP. The non-competitive NMDA antagonist, MK-801, had no effect on the response. The highest dose of amphetamine tested also reduced footshock-induced freezing. However, amphetamine-treated animals did not locomote or rear after footshock, suggesting fear of the environment. Animals injected with DZP, NPC 12626, CPP or buspirone spent at least 1.4 of the 4 post shock minutes locomoting. Haloperidol had no effect on freezing at the doses tested. β-CCE tended to increase the duration of footshock-induced freezing. With the exception of buspirone, none of the compounds which reduced freezing were analgesic in a hot plate test, suggesting that analgesia was not the cause of reduced freezing. Footshock-induced freezing is a simple test which may be useful for detecting the anxiolytic potential of drugs from a number of different classes.

Key words: Anxiety model – Defensive behavior – Anxiolytic – NPC 12626 – Fear behavior

A number of paradigms are available to evaluate the anxiolytic potential of new compounds in animals. Many of these tests are used to examine compound effects on punished responding or conflict behavior. Conflict tests are sensitive to benzodiazepines (for review see Shephard 1986) and it has been reported that buspirone (a serotonin 1A agonist) is active in a conditioned suppression conflict test (McCloosey et al. 1987). However, these procedures require subjects to be food or water deprived and often, extensively trained. Additionally, drug effects on appetitive or consumatory behaviors may confound interpretation of positive results in such paradigms.

A second class of tests involves evaluating rodents’ responsiveness to a novel environment (Treit and Fundytus 1989), or natural avoidance of brightly illuminated (Costall et al. 1987) or open (Pellow et al. 1985; Lister 1987) environments. With these paradigms, non-deprived subjects may be tested. In the elevated plus-maze test for example, the effects of benzodiazepines (Pellow et al. 1985; Lister 1987), barbiturates, ethanol (Lister 1987), and the NMDA receptor antagonist AP7 (Stephens et al. 1986) are detected. However, clinically useful atypical anxiolytics such as buspirone (for review see Ives and Heym 1989), which has reported anxiolytic effects in other animal models (McCloosey et al. 1987; Kehne et al. 1988; Treit and Fundytus 1988), is variably detected as an anxiolytic in the plus-maze test (Pellow and File 1986; but see Soderpalm et al. 1989). Paradigms such as the plus-maze test do not involve the presentation of discrete, “fear-eliciting” stimuli, and thus may represent a more circumscribed model of anxiety than those in which the subject is made fearful by an experimentally programmed event.

A second approach not requiring deprivation or training is to study the effects of drugs on defensive behaviors. Rodents display defensive behaviors upon presentation of stimuli associated with aversive events (Bouton and Bolles 1980) and it has been proposed that defensive behaviors be examined in models of anxiety (Hard et al. 1985; Craft et al. 1988; Fanselow and Helmstetter 1988; Kalin et al. 1988; Treit and Fundytus 1988). In rats, defensive burying of an electrified prod is dimin-
lished by low doses of buspirone and the benzodiazepines (Treit and Fundytus 1988). Freezing (complete immobility), a rodent’s natural defensive reaction to fear-eliciting stimuli (Bolles 1970), is also attenuated by benzodiazepines (Hard et al. 1985; Fanselow and Helmstetter 1988), and by amygdala lesions (Blanchard et al. 1989). Interestingly, both amygdala lesions (Hitchcock and Davis 1987) and anxiolytics such as diazepam and buspirone disrupt fear-potentiated startle (Kehne at al. 1988). Although fear-potentiated startle and conditioned freezing appear to be related responses (Leaton and Borszcz 1985), the effects of non-benzodiazepine anxiolytics have not been systematically examined in a freezing paradigm.

Soon after receiving brief electric footshock, rats assume an easily identifiable freeze posture. In the present experiments, a number of anxiolytics from different chemical classes were examined for their ability to reduce footshock-induced freezing in rats. Diazepam, buspirone and two competitive N-methyl-D-aspartate (NMDA) antagonists decreased the duration of post-shock freezing. Central nervous system acting compounds lacking anxiolytic action (haloperidol and amphetamine) as well as a beta carboline (β-CCE), a reported anxiogenic (for review see File and Baldwin 1987), were also examined. Haloperidol and MK-801 (a non-competitive NMDA antagonist) had no effect on footshock-induced freezing whereas β-CCE tended to increase the duration of the response. The highest dose of amphetamine tested also decreased freezing. However, amphetamine-treated animals did not locomote following the footshock but instead, stood in one place displaying head movement. These data suggest that footshock-induced freezing represents a simple measure of defensive behavior which may be useful for detecting compounds with anxiolytic potential.

Materials and methods

Animals

Male Sprague Dawley rats (Charles River or Harlan Sprague Dawley) 200–350 g were housed in groups of four until 18 h prior to the experiment, when they were transferred to individual housing. Standard rodent chow and tap water were available ad libitum. The colony was maintained on a 14 h light, 10 h dark cycle; lights came on at 0700 hours. All experiments were conducted before 0800 and 1530 hours.

Drugs

The following drugs were prepared in a vehicle of 10% Tween 80 and saline: Diazepam (DZP), dl-amphetamine sulfate (AMP), and haloperidol (HAL), (Sigma, St. Louis); 3-(+/-)-2-carboxypiperazin-4-yl-propyl-1-phosphonic acid (CPP), (Toorais Neuramin, England); ethyl-β-carboline-3 carbboxylate (β-CCE), and [(+)-S-methyl-10,11, dihydroxy-SH-dibenzo(a,d)cyclohepten-5,10-imine (MK–801), (Research Biochemicals Inc., Natick, MA); buspirone hydrochloride was a gift from Bristol Myers; 2-amino-4,5-(1,2-cyclohexyl)-7-phosphonohexoic acid (NPC 12626), (Nova Pharmaceutical Corp., Baltimore, MD); morphine sulfate (MOR) (Lilly, Indianapolis). All drugs were administered intraperitoneally (IP) in 1 ml/kg injections.

Procedure

Footshock-induced freezing. Animals received a single IP injection of test compound or vehicle 30 min prior to being placed in a standard conditioning chamber (Coulbourn Instruments model #E13–10SF) for a 6.5 min session. Two and 2.5 min after the start of the session, a scrambled footshock (0.5 mA, 0.5 s) was delivered through the grid floor of the chamber (Coulbourn Instruments Grid Floor Shocker model #E13–08). Shock delivery and data acquisition were controlled by either a PDP–11 or Compaq computer. Using an assembly of push buttons interfaced with the computer, an observer monitored the amount of time each animal spent engaged in the following mutually exclusive behaviors:

1. Freezing: immobility with rigid body posture.
2. Sedated posture: sitting or sleeping.
3. Small exploratory movements: exploratory movements involving the torso or front paws only, vertical movement of the head, or sniffing.
4. Locomotion: activity involving hind paws, grooming or rearing.

Frequency of rearing was also counted. All behaviors were monitored for the entire 6.5 min session.

Hot plate analgesia test. Compounds which affected freezing were examined in a hot plate analgesia test to assure that freezing was not altered due to a change in pain sensitivity. Mice (CF1-derived, Harlan Sprague Dawley) were injected (IP) with vehicle, morphine (3.0 or 6.0 mg/kg), or the compound of interest prior to being placed on a hot plate which was heated to 54°C. The injection-to-test intervals in minutes for each of the drugs tested were as follows; Mor 15; DZP (2.0–4.0 mg/kg) 30; NPC 12626 (25.0–35.0 mg/kg) 30; CPP (4.0–8.0 mg/kg) 20; BUS (5.0–10.0 mg/kg) 30; AMP (1.0–2.0 mg/kg) 15 or 30; β-CCE (2.5–5.0 mg/kg) 15. Animals were observed for 60 s and the latency to lick a hind paw was recorded.

Data analysis

Duration of time spent in the freeze posture after the second footshock was the primary dependent measure. Data were submitted to a one-way analysis of variance (ANOVA) with dose as a between subjects factor for each of the compounds tested. Results with individual doses were compared to vehicle by a t-test if the ANOVA was significant or if the data suggested that a single dose was effective. Duration of post-shock locomotion, pre-shock rearing and paw lick latencies from the hot plate analgesia test were also submitted to one-way ANOVA.

Results

Figure 1 displays the effects of DZP, NPC 12626, CPP and BUS on the duration of footshock-induced freezing. ANOVA revealed that DZP (2.5 and 5.0 mg/kg) reduced the duration of footshock-induced freezing [F(3,56) = 2.91, P < 0.05]. Similarly, NPC 12626 (12.5 and 25.0 mg/kg) resulted in significantly less post-shock freezing than vehicle, [F(4,35) = 3.03, P < 0.05]. Overall, BUS tended to be effective (P < 0.05, one-tailed test) and 2.5 mg/kg BUS produced an effect which was significantly different from controls (P < 0.05). CPP (5.0 mg/kg) also tended (P < 0.06) to reduce freezing.

Neither HAL nor MK–801 had an effect on freezing at any of the doses tested (Fig 2). There was an overall significant effect of β-CCE, [F(4,32) = 2.87, P < 0.05]: