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BRIEF COMMUNICATION

Excitatory Effects of the Vasodilator Hydralazine on Acoustic Startle in the Rat

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COMMISSARIS, R. L. AND M. DAVIS. *Excitatory effects of the vasodilator hydralazine on acoustic startle in the rat.* PHARMACOL BIOCHEM BEHAV **19**(5) 891–894, 1983.—Several drugs that functionally depress noradrenergic transmission depress acoustic startle amplitude (e.g., clonidine, phenoxybenzamine). Since these agents also depress blood pressure, the current study was designed to investigate the effects of hypotension *per se* (in the absence of decreased noradrenergic transmission) on the acoustic startle response. The vasodilator hydralazine was chosen because it produces marked hypotension in conscious rats, yet results in a compensatory increase in noradrenergic transmission (sympathetic rebound), rather than a decrease, as is seen with many other hypotensive agents. Hydralazine (0.3–20 mg/kg, IP) produced a marked and long-lasting increase in startle amplitude, compared to saline-treated controls. In a similar group of subjects, IP administration of 2.5 mg/kg hydralazine decreased mean arterial blood pressure by about 50% in conscious rats. Since, at the doses and treatment times employed in the present study, hydralazine decreases blood pressure but not startle amplitude, these data suggest that there is no relationship between changes in blood pressure and changes in startle amplitude across all drugs. Moreover, since hydralazine-induced hypotension results in an increase in noradrenergic transmission (sympathetic rebound), these data are consistent with the hypothesis that increases in central noradrenergic transmission increase acoustic startle amplitude. Lastly, since the most prominent direct action of hydralazine occurs in the periphery, hydralazine may alter startle through central actions that are triggered in the periphery.

Hydralazine Acoustic startle Blood pressure Norepinephrine

DRUGS which decrease noradrenergic transmission (e.g., clonidine, alpha-1 adrenergic antagonists) have been shown to reduce the amplitude of the acoustic startle reflex [3]. These drugs are thought to exert their effects by directly altering central noradrenergic modulation of the startle reflex. However, in addition to affecting startle, these drugs also have pronounced hypotensive effects. In fact, it could be argued that the drug-induced decreases in blood pressure are in some way responsible for the observed decreases in startle amplitude.

One pharmacological approach for testing this hypothesis would be to evaluate the effects of a drug on startle which produces hypotension without decreasing central noradrenergic transmission. The antihypertensive agent hydralazine was chosen for study, since its potent hypotensive effects appear to be mediated by direct dilatatory action on vascular smooth muscle [5, 6, 9], and are associated with a compensatory increase (not decrease) in central noradrenergic activity [1]. If hydralazine administration depressed startle amplitude at doses which depressed blood pressure, this would suggest that hypotension *per se* is a condition sufficient to depress the startle reflex. On the other hand, if hydralazine did not depress startle, this would provide negative evidence for a simple relationship between changes in blood pressure and changes in startle amplitude. Finally, if hydralazine actually increased startle, this would provide further evidence for a relationship between activation of noradrenergic transmission and excitation of startle.

METHOD

Subjects

Male Sprague-Dawley rats (Charles River Co.) weighing 350–450 g were used. All animals were housed in groups of four or five with a 12 hour light-dark cycle (lights on 0700–1900 hr). All subjects had food and water ab lib.

Startle Apparatus

The apparatus used to measure startle has been described previously [10]. Briefly, 5 separate stabilimeters were used

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to record the amplitude of the startle response. Each stabilimeter consisted of an 8×15×15-cm Plexiglas® and wire mesh cage suspended between compression springs within a steel frame. Cage movement resulted in displacement of an accelerometer where the resultant voltage was proportional to the velocity of displacement. Startle amplitude was defined as the maximum accelerometer voltage that occurred during the first 200 msec after the startle stimulus was delivered and was measured with a specially designed sample-and-hold circuit. The stabilimeters were housed in a dimly lit, ventilated, sound attenuated chamber. Each startle stimulus was a 90 msec, 115-dB burst of white noise (rise-decay time of 5 msec) delivered through a high-frequency speaker (Radio Shack-Super Tweeter®) located 15 cm behind each test cage. Background white noise, provided by a white noise generator, was 55 dB. Sound level measurements were made within the cages with a General Radio Model 1551-C sound level meter (A-scale).

Startle Testing Procedure

Each group of rats was tested on two occasions. Each test session consisted of repetitive presentation of the startle stimulus at a 20 sec interstimulus interval. For each test session the rats were placed in the startle cages and 5 sec later exposed to startle-eliciting noise bursts for 14 min (42 noise bursts) to establish a pre-injection baseline. The rats were then removed from their cages and injected with either saline or one of the hydralazine (Sigma Chemical Co., St. Louis, MO) doses employed in the present study (0.31–20.0 mg/kg). Immediately following injection the rats were returned to the test cages and the startle-eliciting stimuli were presented for an additional 90 min (270 noise bursts). At least two days later this procedure was repeated, except that animals which had received saline injections on the first day were given hydralazine and those which had received hydralazine on test day 1 were injected with saline. Each rat therefore served as his own control with respect to saline and hydralazine, while dosage varied between rats.

Blood Pressure Measurements

Animals were anesthetized with 400 mg/kg chloral hydrate; a hybrid PE 10/PE 20 catheter was then inserted into the femoral artery and "snaked" toward the heart to terminate in the abdominal portion of the descending aorta. The catheter was then secured to the femoral artery and the PE 20 portion was guided under the skin to protrude from the back of the neck. The animals were allowed to recover for two days prior to testing.

The testing procedure for blood pressure measurements was the same as that employed in the startle experiment. On the first test day one half of the subjects received saline and the other half received 2.5 mg/kg hydralazine, IP. Two days later, the treatments were reversed. Each test day consisted of a fourteen minute baseline period, followed by IP injection and an additional 90 minutes of blood pressure monitoring. The transducer used for blood pressure measurements was a Stratham P23Gb transducer connected to a Gould Bruschart recorder.

RESULTS

The left panel of Fig. 1 illustrates the effects of saline and 2.5 mg/kg hydralazine on the amplitude of the acoustic startle response. While saline injection had no effect on startle amplitude, treatment with 2.5 mg/kg hydralazine markedly

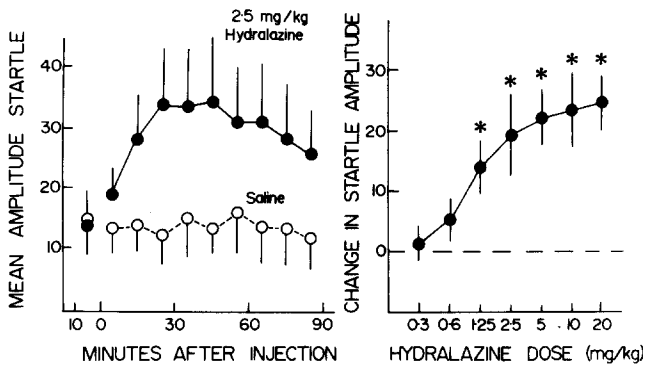

FIG. 1. Left panel: Time course for the effects of hydralazine on the acoustic startle response. The effects of IP saline (open circles) or 2.5 mg/kg hydralazine (filled circles) on the amplitude of the acoustic startle response are plotted for various times after injection. Each symbol and vertical bar represents the mean±S.E.M. obtained from nine animals. Right panel: Dose response effect of hydralazine on the acoustic startle response. Each point represents the net change in startle amplitude produced by various doses of hydralazine. Net hydralazine effect was determined by comparing the change in startle 30–60 minutes following hydralazine administration (relative to pre-injection baseline) to the change in startle following saline administration (30–60 minutes post-injection compared to baseline). Each symbol and vertical bar represents the mean±S.E.M. from nine or ten animals. *$p<0.05$, paired t-test.

increased startle amplitude above baseline (pre-injection) levels. This effect of hydralazine, although apparent within the first 10 min after injection, did not become maximal until approximately 20–30 min after injection at which time startle was elevated by about 100 percent. Startle amplitude remained elevated for about 30–60 min post-injection and then declined slightly over the remainder of the session. This delayed onset of action was observed with all doses of hydralazine tested.

The right panel of Fig. 1 illustrates the dose-response effect of hydralazine on startle expressed as the change in amplitude over a 30–60 min period after drug injection relative to saline injection. Hydralazine produced a dose-dependent increase in startle amplitude with a threshold of approximately 1.25 mg/kg. A maximal effect was obtained at 20 mg/kg. Analysis of variance (ANOVA) revealed a significant dose effect, $F(6,61)=3.63$, $p<0.01$; trend analysis indicated a significant linear trend, $F(1,61)=20.77$, $p<0.01$.

Previous studies have shown that, under certain conditions, the startle reflex may increase in amplitude over the course of repetitive stimulus presentations [3,4]. To test whether the delayed onset observed with hydralazine was due to a delayed onset of the drug effect alone or to an interaction of the drug with repeated stimulus presentations (sensitization), a group of rats received saline or 20 mg/kg hydralazine following a baseline period and were then returned to their home cages for 60-min before initiation of the post-injection trials (60-min test-retest interval). The data from these animals were compared to data from subjects receiving 20 mg/kg hydralazine and saline using a standard 1-min test-retest interval. The left panel of Fig. 2 illustrates the lack of effect of hydralazine for the first five noise bursts after injection in the 1-min test-retest interval condition. The right panel of Fig. 2 illustrates that the startle-increasing effect of hydralazine in the 60-min test-retest interval group was apparent immediately (first noise burst) upon the return of the animals to their test cages and was not dependent upon

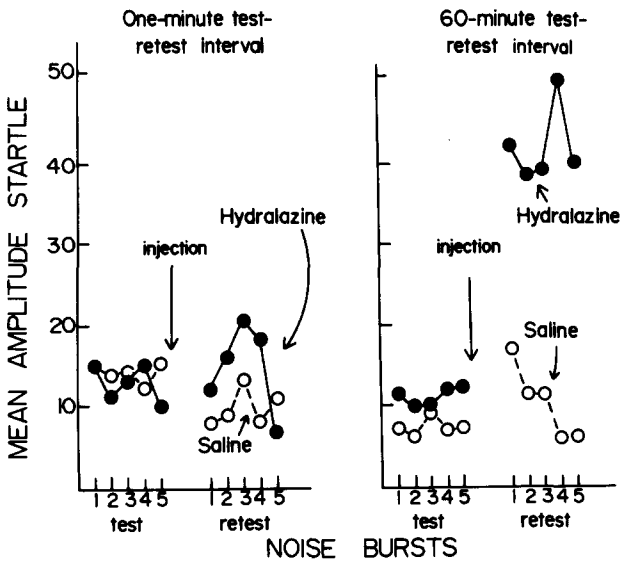

FIG. 2. The effects of 20 mg/kg hydralazine on startle amplitude one minute or 60 minutes after injection. Mean amplitude startle is plotted for the last five noise bursts before (test) and the first five noise bursts after (retest) injection of saline (open circles) or 20 mg/kg hydralazine (filled circles). The left panel illustrates the lack of effect using a one-minute test-retest interval; the right panel illustrates the immediate and maximal effect using a 60-minute test-retest interval. Each point represents the mean obtained from five (one-minute) or nine (60-minute) rats.

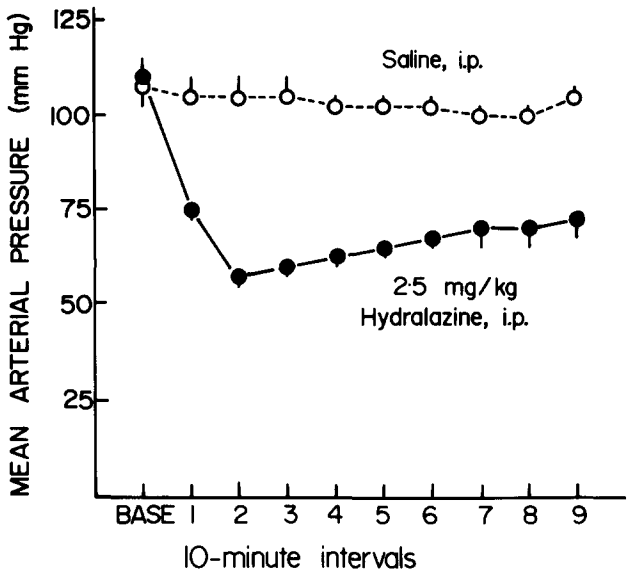

FIG. 3. The effects of hydralazine on arterial blood pressure in conscious rats. The effects of IP saline (open circles) or 2.5 mg/kg hydralazine (filled circles) on aortic arterial blood pressure are plotted for various times after injection. Each symbol and vertical bar represents the mean±SEM from four animals.

repeated stimulus presentations or exposure to the background noise in the test chamber. Factorial ANOVA comparing the change in startle amplitude from the average of the last five noise bursts before injection to the average of the first five bursts after injection revealed significant effects for hydralazine vs. saline, $F(1,12)=18.29$, $p<0.01$, 1-min vs. 60-min test-retest intervals, $F(1,12)=24.24$, $p<0.01$, and a significant drug by test-retest interval interaction, $F(1,12)=7.85$, $p<0.05$.

Figure 3 illustrates the time course for the effects of 2.5 mg/kg hydralazine on blood pressure in conscious rats. As can be seen, hydralazine dramatically reduced blood pressure relative to saline injection; this effect was apparent within the first 10 minutes post-injection, reached a maximum within 20 minutes post-injection, and persisted throughout the 90-minute post-injection period.

DISCUSSION

In the present study, hydralazine produced both a decrease in blood pressure and an increase in acoustic startle amplitude. The increase in startle amplitude was large, representing 108 and 138 percent increases at the 10 and 20 mg/kg doses, respectively. Although the onset of the startle-increasing effect of hydralazine was delayed, this delay was found not to relate to an interaction with sensitization produced by repeated stimulus presentations or background noise. Interestingly, the lowest dose for producing a significant increase in startle amplitude in the present study, 1.25 mg/kg, IP, is approximately the threshold dose for decreasing blood pressure in conscious rats (2,8; Commissaris and Davis, unpublished). Since other drugs which are known to depress blood pressure actually depress startle amplitude (e.g., phenoxybenzamine, clonidine), it would appear that there is no correlation between these two measures across all drugs.

Recent reports have indicated that decreases in blood volume [7] or blood pressure [1] result in increases in brain noradrenergic activity and turnover. Since other treatments which increase noradrenergic transmission also increase startle amplitude [3], it is possible that the startle-increasing effects of hydralazine might result from a compensatory (triggered by hypotension) increase in noradrenergic activity. The relatively delayed onset for the effects of hydralazine on both blood pressure and behavior is consistent with such an hypothesis. Also consistent with this hypothesis are preliminary data indicating that the startle-enhancing effects of hydralazine are attenuated by pretreatment with the alpha-1-antagonist prazosin and the alpha-2 agonist clonidine (Commissaris and Davis, unpublished).

Lastly, unlike many other agents which affect startle amplitude, the most prominent direct action of hydralazine is peripheral in nature [5,9], with changes in central sympathetic activity occurring secondarily to changes in the periphery (i.e., vasculature; [6]). Thus, hydralazine may alter startle through compensatory central actions that are triggered initially outside the central nervous system.

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NOTE ADDED IN PROOF

In addition to the present findings, recent work by Leaton *et al.* (*Physiol Behav* **31:** 103–109, 1983) has indicated that spontaneously-hypertensive rats (SHRs) have *decreased* startle amplitudes relative to normotensive controls (WKYs), further supporting the lack of correlation between these two measures.