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TRICYCLIC ANTIDEPRESSANTS VARY IN DECREASING α_2-ADRENOCEPTOR SENSITIVITY WITH CHRONIC TREATMENT: ASSESSMENT WITH CLONIDINE INHIBITION OF ACOUSTIC STARTLE

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1 Clonidine inhibition of the acoustic startle reflex in the rat was used as a behavioural measure of α_2-adrenoceptor sensitivity following acute or chronic administration of tricyclic antidepressants.

2 Chronic (14 day) administration of desipramine (10 mg/kg, i.p.) attenuated the depressant effect of clonidine (20 or 40 μg/kg) on the startle reflex.

3 No change in response to clonidine was obtained after chronic treatment with two other tricyclic antidepressants, amitriptyline (10 mg/kg) or iprindole (5 mg/kg).

4 Acute administration of these tricyclics (1 h) did not modify the effect of clonidine on startle.

5 It is suggested that the development of α_2-adrenoceptor subsensitivity produced by chronic tricyclics may be unique to those compounds, such as desipramine, which are active in blocking the uptake of noradrenaline.

Introduction

Therapeutic effects of the tricyclic antidepressants (TCAs) typically do not appear until two or more weeks after the start of treatment (Oswald, Brezinova & Dunleavy, 1972; Klein, Gittelman, Quitkin & Rifkin, 1980). Hence the neuropharmacological actions of the TCAs that accompany chronic as opposed to acute administration should be the ones most relevant to their therapeutic effects. Chronic, but not acute, administration of TCAs decreases the density and functioning of β-adrenoceptors (Wolfe, Harden, Sporn & Molinoff, 1976; Banerjee, Kung, Riggi & Chanda, 1977) while increasing responsiveness of α_1-adrenoceptors and 5-hydroxytryptamine (5-HT) receptors in brain (de-Montigny & Aghajanian, 1978, Menkes, Aghajanian & McCall, 1980; Wang & Aghajanian, 1980; Menkes & Aghajanian, 1981). It should be noted that changes in each of these receptor systems are seen after chronic treatment with a variety of TCAs that differ in their acute presynaptic actions (cf., Charney, Menkes & Heninger, 1981). Recently, several reports have suggested that chronic but not acute treatment with certain TCAs decreases the functional sensitivity of α_2-adrenoceptors in brain (Svensson & Usdin, 1978; Tang, Helmeste & Stancer, 1978; Tang, Helmeste & Stancer, 1979; McMillen, Warnack, German & Shore, 1980; Spyraki & Fibiger, 1980; Sugrue, 1981). This conclusion was based on the observation that some chronic TCAs decrease behavioural, electrophysiological, and biochemical responses to the α_2-adrenoceptor agonist, clonidine. To date, however, it is not clear whether this effect is common to all TCAs or restricted to those, such as desipramine (DMI) or imipramine, which are active in blocking the reuptake of noradrenaline (NA). Moreover, changes in brain α_2-adrenoceptor binding after TCA treatment are controversial, with reports of increases, decreases, and no change in the number of [^{3}H]-clonidine binding sites (Johnson, Reisine, Spotnitz, Weich, Ursillo & Yamamura, 1980; Reisine, U'Prichard, Ursillo & Yamamura, 1980; Peroutka & Snyder, 1980; Vetulani, Pik & Nikitin, 1980; Smith, Garcia-Sevilla & Hollingsworth, 1981; Tang, Seeman, & Kwan, 1981).

The startle reflex in the rat is greatly depressed by low doses of the α_2-agonist, clonidine (Davis, Cedarbaum, Aghajanian & Gendelman, 1977; Geyer, Petersen, Rose, Horwitt, Light, Adams, Zook, Hawkins & Mandell, 1978). This effect appears to be mediated by α_2-adrenoceptors since it can be blocked by pretreatment with the α_2-antagonists yohimbine or piperoxane, but not by α_1-adrenoceptor or 5-HT antagonists (Davis *et al.*, 1979; Davis & Astrachan, 1981). Changes in acoustic startle after clonidine should provide, therefore, a sensitive behavioural

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test system to evaluate alterations in central α_2-sensitivity. Accordingly, this study was designed to evaluate whether chronic administration of clinically effective TCAs would alter the depressant effect of clonidine on startle.

Methods

Male albino rats (Charles River Co.) weighing 300–350 g were housed five to a cage and maintained on a 12 : 12 hour light : dark schedule (lights on at 07 h 00 min). Food and water were available *ad libitum*. The apparatus used to measure startle is described by Weiss & Davis (1976). Briefly, five separate stabilimeters were used to record the amplitude of the startle response. Each stabilimeter consisted of an 8 by 15 by 15-cm Plexiglas and wire mesh cage suspended between compression springs within a steel frame. Cage movement caused displacement of an accelerometer; the resultant voltage was proportional to the velocity of displacement. Startle amplitude was defined as the maximum accelerometer voltage during the first 200 ms after the stimulus and was measured with a sample-and-hold circuit. The stabilimeters were housed in a dimly lit, ventilated, sound-attenuated chamber, 1.1 mm from a high-frequency speaker. The startle stimulus was a 90 ms, 115 dB burst of white noise with a rise-decay time of 5 ms. Background white noise, provided by a white noise generator, was 55 dB. Sound level measurements were made in the cages with a General Radio Model 1551-C sound level meter (A-scale).

In the first experiment a total of 60 rats was placed in the startle cages and presented with 10 noise bursts at 20 s intervals. The rats were then divided into three equal groups with equivalent mean startle amplitudes. Animals in each group ($n = 20$) were injected intraperitoneally (i.p.) once a day for 14 days with saline, desipramine HCl (DMI, 10 mg/kg, USV Pharmaceuticals), or amitriptyline (Ami, 10 mg/kg, Merck, Sharpe & Dohme). This dose was used since it has been shown to alter functional sensitivity in a number of receptor systems (cf., Charney *et al.*, 1981). All drug weights are based on the weight of the salt. Twenty-four hours after the last injection five rats in each group were injected with saline and five with clonidine (20 μg/kg). Immediately afterwards they were placed into the startle test cages and presented with a total of 90 noise bursts at 20 s intervals, constituting a 30 min test session. Twenty-four hours later the same procedure was repeated except rats previously given saline were now given clonidine (20 μg/kg) and vice versa. The other rats in each of the groups were treated identically except here the dose of clonidine was 40 μg/kg. Thus, each rat served as its own control with respect to saline vs. clonidine, whereas the dose of clonidine and pretreatment TCA varied across different rats. All rats were given their usual TCA or saline injection immediately after the Day 1 test session, so that testing with clonidine occurred 24 h after the last TCA injection on both the first and second test day. These exact procedures were repeated on another set of 60 rats. Two groups of 20 rats each received saline or DMI to replicate the previous results. The other group received the atypical TCA iprindole (Ipr, 5 mg/kg, Wyeth Labs). This dose of Ipr was used because of its greater clinical potency relative to the other TCAs (El-Deiry, Forrest & Littmann, 1967).

To determine if any of the TCAs might alter the effect of clonidine on startle after acute administration, an additional 40 rats were pretested and matched into four groups of 10 rats each. Two days after matching, rats received injections of either saline, DMI 10 mg/kg, Ami 10 mg/kg, or Ipr 5 mg/kg. One hour later half the rats in each group were injected with saline and half with clonidine (40 μg/kg) and then tested for startle over the next 30 min as described earlier. Twenty-four hours later the same TCA treatments were repeated and one hour later the rats were given clonidine or saline. In this case, however, rats previously given clonidine were given saline and vice versa. Finally, another 30 rats were pretested and matched into three groups of 10 rats each. Identical procedures to those described immediately above were then carried out, except in this case rats were acutely pretreated with either saline, or DMI 5 or 15 mg/kg.

Results

Figure 1 shows the mean amplitude startle response averaged over successive 2 min periods after injections of saline or 20 or 40 μg/kg clonidine in the four pretreatment conditions. Consistent with earlier work, clonidine depressed acoustic startle, with greater depression at 40 vs. 20 μg/kg. Most important, however, was that the magnitude of this depression was reduced in the rats chronically pretreated with DMI but not in those given chronic Ami or Ipr. To evaluate these effects, an overall analysis of variance was performed, using pretreatment drug (e.g., saline, DMI, Ami, or Ipr) and clonidine dose (20 or 40 μg/kg) as between subject factors and trial blocks and clonidine (vs. saline) as within subject factors. Overall, there was a significant clonidine effect, $F(1,108) = 319.74$, $P < 0.001$, reflecting the depression of startle caused by clonidine, as well as a significant clonidine by dose interaction, $F(1,108) = 5.12$, $P < 0.02$, reflecting a greater depression at 40 vs. 20 μg/kg. There was also a significant trials effect $F(14,1512) = 54.19$, $P < 0.001$, reflect-

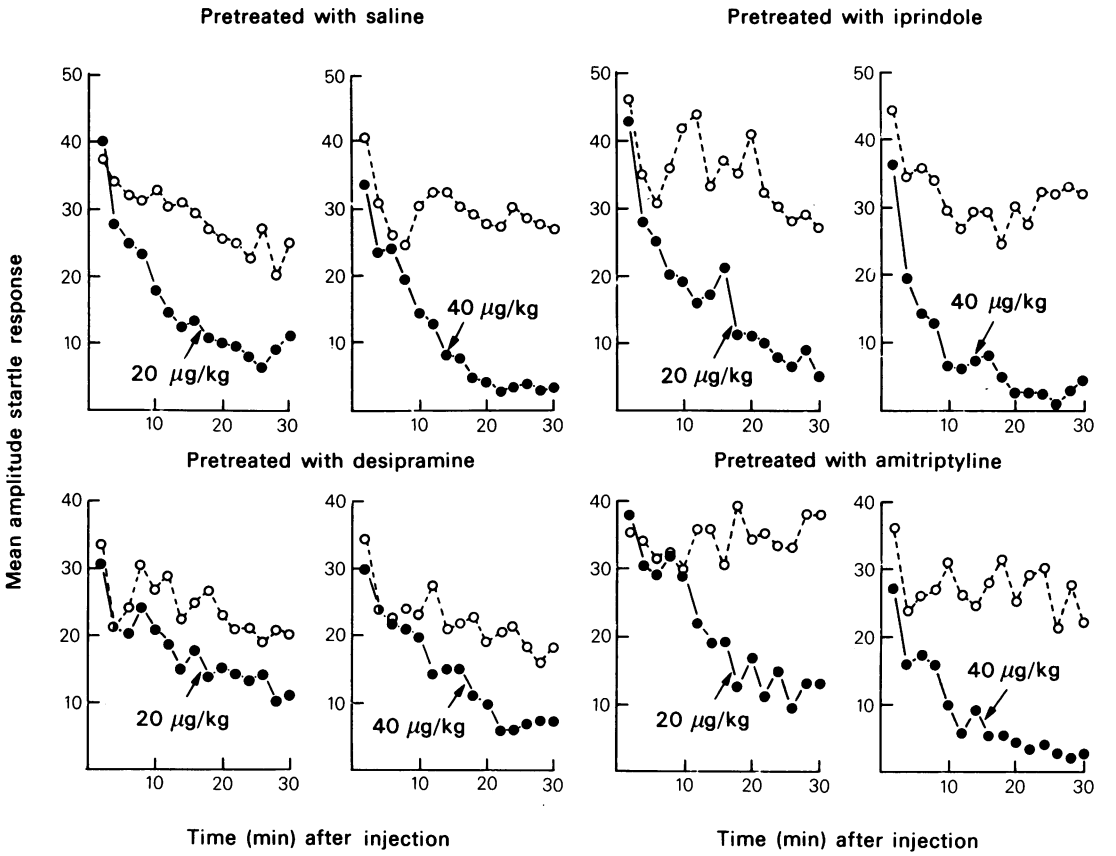

Figure 1 Mean amplitude startle responses at various times after injection of saline (○) or 20 or 40 μg/kg clonidine (●) in rats pretreated for 14 days with saline, desipramine (10 mg/kg), iprindole (5 mg/kg) or amitriptyline (10 mg/kg).

ing the general decrease in startle across the test session, as well as a significant clonidine by trials interaction, F (14,1512) = 18.91, $P<0.001$, reflecting the faster rate of startle decrease following injection of clonidine vs. saline. Of major importance, however, was a significant pretreatment drug and clonidine interaction, F (3,108) = 12.34, $P<0.001$, indicating that the magnitude of the clonidine effect on startle differed across the various pretreatment conditions. Visually, this interaction would reflect the smaller depression by clonidine in the rats pretreated with DMI compared to the other groups. Consistent with this, subsequent individual comparisons indicated that the magnitude of the depressant effect of clonidine at either 20 or 40 μg/kg was significantly smaller after pretreatment with DMI than with the other pretreatments ($P<0.01$ in every case). In contrast, the magnitude of the clonidine effect did not differ significantly among the other pretreatments.

Overall, the baseline startle amplitudes were somewhat lower in the DMI-treated rats compared to the other groups. Since this by itself would tend to reduce the magnitude of the clonidine effect when absolute change scores are compared, the data were also analyzed in terms of percentage change scores. The period from 10 to 20 min after injection was used since this seemed to be the most sensitive test period to detect differences in the effect of clonidine on startle. These results are illustrated in Figure 2 and indicate that the magnitude of the depressant effect of clonidine was less in DMI-treated rats at both test doses of clonidine, even when the data were analyzed in terms of percentage change scores. For example, clonidine depressed startle in rats pretreated with saline by about 55% after 20 μg/kg and about 75% after 40 μg/kg. In contrast, the same doses of clonidine depressed startle by about 30 or 45% in the rats pretreated with DMI and the differences between the percentage depression between saline and

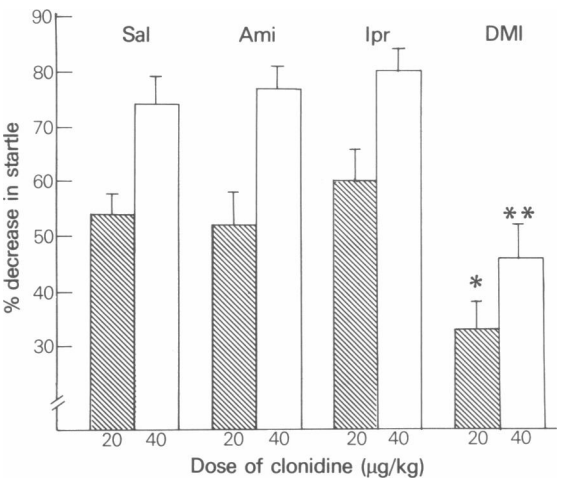

Figure 2 Percentage decrease in startle after injection of either 20 or 40 μg/kg clonidine HCl in rats pretreated for 14 days with saline (Sal), amitriptyline (Ami) (10 mg/kg), iprindole (Ipr) (5 mg/kg) or desipramine (DMI) (10 mg/kg); vertical lines indicate s.e. Change in startle is computed as a percentage of saline baseline ((saline minus clonidine)/saline × 100) and was calculated for each chronic treatment group; baseline means were not significantly changed by the chronic treatments (29.6, 28.8, 33.0, and 23.9 for the chronic saline, Ami, Ipr, and DMI groups respectively, F' (3/74) = 0.24 $P > 0.10$. * $P < 0.01$ relative the 20 μg dose in rats pretreated with saline or Ami or Ipr. ** $P < 0.01$ relative to the 40 μg dose in rats pretreated with saline or Ami or Ipr.

DMI pretreated rats was significant at each test dose of clonidine, t (37) = 2.79, $P < 0.01$ for 20 μg/kg; t (37) = 3.29, $P < 0.01$ for 40 μg/kg. The attenuated response to clonidine after DMI occurred in both replications and was significant in each case.

In contrast to DMI, Figure 2 shows that chronic treatment with either Ami or Ipr did not alter the depressant effect of clonidine on acoustic startle. After these TCAs, the percentage changes in startle after either 20 or 40 μg/kg of clonidine were essentially identical to that observed after saline and significantly greater than the percentage changes after DMI, ($P < 0.01$ at each dose when DMI was compared with Ami or with Ipr).

In contrast to chronic DMI treatment, acute treatment with DMI or the other TCAs did not significantly affect the response to clonidine relative to that observed after acute saline (percentage decreases = 80.0, 94.3, 88.9, and 77.4 after Ipr, Ami, DMI or Sal respectively, F (3/36) = 2.03, $P < 0.10$). Using the same doses as in the chronic study, none of the TCAs significantly altered baseline startle levels after acute administration (baseline means = 32.4, 29.8, 30.7, or 32.4 for Ipr, Ami, DMI and Sal respectively = F(3/36) = 0.78, $P > 0.10$).

Finally, acute administration of either 5 or 15 mg/kg DMI did not significantly attenuate the effects of clonidine on startle (percentage decreases = 86.2, 76.0, and 70.8 after 5, 15 mg/kg DMI or saline, respectively, $P > 0.10$. It is noteworthy that even though the high dose of 15 mg/kg did significantly reduce the baseline startle response (by about 50%), it did not interfere with clonidine's effect on startle. Thus, it is unlikely that the attenuation of clonidine's effect by chronic DMI can be explained by the slight decrease (about 20%) in baseline startle in this condition.

Discussion

These results indicate that chronic but not acute treatment with DMI attenuates the depressant effect of clonidine on acoustic startle. Since clonidine appears to depress startle via an α_2-adrenoceptor, the results suggest that chronic DMI leads to a decrease in α_2-adrenoceptor sensitivity, consistent with previous biochemical, electrophysiological, and behavioural studies (see Introduction). Although a pharmacokinetic explanation cannot be ruled out, brain accumulation of systemic [^{3}H]-clonidine is known to be unaffected by chronic treatment with another TCA, Ami (Greenberg, Curro, Palmer, Palmer, Darda & Hoefke, 1980). An alternative possibility, that chronic DMI decreases clonidine's effect because of α_2-adrenoceptor blockade, is unlikely for two reasons. First, the effect of clonidine was not altered 1 h after an acute dose, despite the fact that brain levels of DMI are known to be higher at this time than 24 h after the last injection of a chronic sequence (Vetulani, Stawarz, Dingell & Sulser, 1976). Second, DMI has a relatively low affinity for [^{3}H]-clonidine binding sites, lower in fact than Ami (Tang & Seeman, 1980) which fails to affect clonidine's depressant effect on startle with either acute or chronic treatment. Thus, it appears that the effect of chronic DMI is not related to a direct intraction with α_2-adrenoceptors.

In contrast to the effect of DMI, chronic administration of two other clinically effective TCAs (Ami and Ipr), at doses that are known to alter other receptor systems, did not modify the effect of clonidine. This difference in the chronic effects of various TCAs on α_2-sensitivity may be related to the varying affinity these drugs have for a presynaptic uptake system for NA. DMI is a potent blocker of NA uptake (Glowinski & Axelrod, 1964; Sulser & Sanders-Bush, 1971) and may, with chronic treatment, produce persistent elevation of synaptic NA and α_2-receptor stimulation. Since manipulations

that increase catecholamine receptor stimulation generally result in receptor desensitization (Schwartz, Costentin, Martres, Protais & Baudry, 1978), it is not surprising that chronic DMI might lead to α_2-subsensitivity. On the other hand, Ami and Ipr, which are less active NA uptake blockers (Glowinski & Axelrod, 1964; Sulser & Sanders-Bush, 1971; Ross, Renyi & Ogren, 1971), might not be expected to do so. Single-unit physiological studies have also provided evidence that TCAs active in blocking the reuptake of NA may promote α_2-subsensitivity with chronic treatment while others, such as chlorimipramine, may not share this property (Svensson, 1980). Biochemical assessment of functional α_2-sensitivity in brain has also been made possible by measuring clonidine-induced decrements in the NA metabolite 3-methoxy-4-hydroxyphenylglycol (MHPG). With this technique, too, the attenuated response to clonidine after chronic DMI appears not to be reproduced by TCAs which are less active in blocking NA uptake (Tang *et al.*, 1978; Sugrue, 1981). Taken together, the data suggest that a change in α_2-adrenoceptor sensitivity may not be relevant to the therapeutic effects of TCAs, since this property is not shared by clinically effective drugs of this class which fail to affect NA uptake significantly. Nonetheless, effects on α_2-sensitivity may be of clinical interest inasmuch as a subgroup of depressives with low MHPG may respond preferentially to TCAs active in blocking NA uptake (Maas, 1975). Finally, these results indicate that clonidine suppression of startle provides a sensitive behavioural measure of functional changes in central α_2-adrenoceptor sensitivity.

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