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Tolerance to Amphetamine-Induced Inhibition of Neuronal Activity in the Central Amygdaloid Nucleus

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REBEC, G. V. AND E. H. LEE. *Tolerance to amphetamine-induced inhibition of neuronal activity in the central amygdaloid nucleus.* PHARMACOL BIOCHEM BEHAV 19(2) 219–223, 1983.—Rats were pretreated twice daily for 5 consecutive days with saline or 2.5 mg/kg d-amphetamine. Approximately 12 hr after the last injection, neuronal activity was recorded bilaterally from the central amygdaloid nucleus (CAN) and the animals were challenged every 2 min with 0.2 mg/kg d-amphetamine or with increasing incremental doses of apomorphine. In saline controls, all CAN neurons were inhibited by the 5th amphetamine injection and two-thirds were suppressed by 0.64 mg/kg apomorphine. In amphetamine-pretreated animals, on the other hand, the majority of CAN neurons failed to respond even by the 10th amphetamine injection and less than one-third were inhibited by apomorphine even at a dose of 2.56 mg/kg. These results indicate that tolerance develops to the inhibitory effects of d-amphetamine in the CAN and that this effect is mediated, at least in part, by a decrease in the sensitivity of postsynaptic dopamine receptors.

d-Amphetamine Apomorphine Central amygdaloid nucleus Long-term treatment Tolerance Unit activity

REPEATED injections of amphetamine produce a progressive augmentation of behavior that includes an increase in forward locomotor activity, sniffing, and repetitive head movements [10, 33–35]. The mesotelencephalic dopamine (DA) system, which projects to the neostriatum and other forebrain sites, has been implicated in all these behaviors [6, 17, 29] and, accordingly, the response of neostriatal neurons to amphetamine is enhanced in rats exposed to long-term amphetamine treatment [1, 20, 31]. Moreover, the neuronal response to apomorphine, a DA receptor agonist, is also enhanced in these animals [31]. Thus, the enhanced sensitivity of neostriatal neurons to DA agonists may mediate, at least in part, the behavioral augmentation that accompanies multiple amphetamine injections.

Tolerance, however, develops to many of the behavioral effects of amphetamine including anorexia, sympathetic arousal, and stereotyped licking and biting [10, 15, 19, 22, 33, 34]. Interestingly, the central amygdaloid nucleus (CAN) appears to exert a modulatory influence on all these responses. Lesions or stimulations of this site, for example, have been reported to alter food intake [4, 16, 26], blood pressure [9, 14, 37], and the expression of amphetamine-induced oral stereotypy [5, 7, 8]. It is conceivable, therefore, that unlike the neostriatum, neurons in the CAN may develop tolerance to the actions of amphetamine. Thus, in the present series of experiments we extended our electrophysiological analysis to the CAN following acute or long-term amphetamine administration. In addition, because the CAN receives DA input, separate groups of animals were challenged with apomorphine to examine a possible change in DA receptor sensitivity.

METHOD

Male, Sprague-Dawley rats (approximately 300 g) were pretreated twice daily with subcutaneous (SC) injections of saline or 2.5 mg/kg d-amphetamine sulfate (Smith, Kline and French) for 5 consecutive days. This schedule allowed sufficient time for the above-described behavioral alterations to develop [33,34]. Body weights of both groups of animals were comparable at the end of the pretreatment period. Approximately 12 hr after the last injection, each animal was secured in a stereotaxic frame under ether anesthesia and prepared for single unit recordings as previously described [30]. Briefly, holes were drilled bilaterally in the skull overlying the CAN, approximately 4.4 mm anterior and 3.4 mm lateral to stereotaxic zero [21]. All points of surgical and stereotaxic contact were thoroughly infiltrated with local anesthetics (Procaine and Xylocaine) and the ether was withdrawn. The animal was immobilized with tubocurare (Lilly) and artificially respired. Heart rate, body temperature (37 ± 0.5°C), and endtidal carbon dioxide (4.0 ± 1.0%) were monitored continuously throughout the experiment. Recordings of electrocorticographic activity in similarly prepared animals were dominated by slow-wave activity indicating effective local anesthesia [30]. Tungsten microelectrodes, having an impedance of 5–10 MΩ, were lowered into the CAN and the search was begun for spontaneously active neurons having a signal-to-noise ratio of 3:1 or more. Following the isolation of single unit discharges, firing rate, displayed on an oscilloscope screen and counted by a high-speed printer-counter, was recorded for a minimum of 20 min and plotted at 15-sec intervals. The mean firing rate/interval was defined as the baseline rate of 100%. Each animal received intrave-

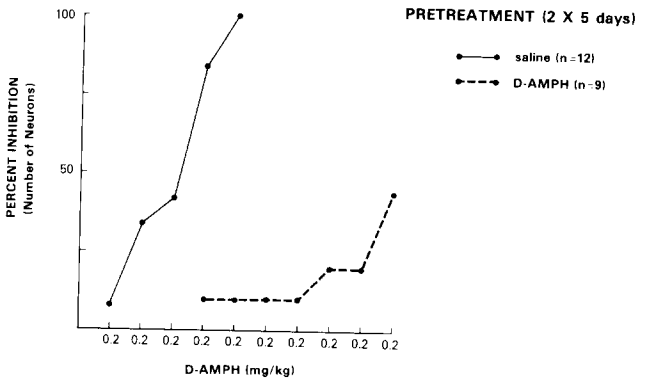

FIG. 1. Percentage of CAN neurons inhibited by IV challenge injections of d-amphetamine following twice daily SC injections of either saline or 2.5 mg/kg d-amphetamine for 5 consecutive days. Note that whereas the 5th challenge injection of 0.2 mg/kg D-AMPH depressed the firing rate of all control neurons (n=12), less than half the neurons (4 of 9) in rats pretreated with d-amphetamine were inhibited even by the 10th challenge injection.

nous (IV) challenges of either d-amphetamine or apomorphine hydrochloride (Merck) via a previously implanted jugular catheter. The d-amphetamine was administered every 2 min at a challenge dose of 0.2 mg/kg until unit activity changed by at least 50% from the baseline rate or until the 10th injection. Apomorphine was administered at 2-min intervals in increasing incremental doses beginning with 0.0025 mg/kg and ending with 0.64 mg/kg [32]. Drug-induced changes in firing rate were plotted as a percentage of the 100% baseline rate. All drug dosages were expressed as the free base. Each rat received only one series of challenge injections (either d-amphetamine or apomorphine) to avoid any residual effects associated with drug accumulation. In some cases, firing rate failed to change or was depressed for a prolonged period following the challenge injections. When either of these events occurred, 10.0 mg/kg clozapine was injected (IV) to demonstrate that the neuron was still responsive [28]. Neurons that failed to maintain a constant signal-to-noise ratio or that failed to return to the baseline rate were not included in the analysis.

Upon completion of the experiment, each animal received an overdose of sodium pentobarbital (Nembutal) and current was passed through the electrode to make a small lesion. Methylene Blue was injected through the catheter and the venous system subsequently inspected for the presence of dye to insure an accurate IV injection. Following a transcardial perfusion with normal saline and 10% Formalin, the brain was removed, sectioned and stained with cresyl violet.

RESULTS

Histological analysis revealed that 44 neurons were recorded from the CAN. Firing rate in both saline and amphetamine-pretreated animals was slow and irregular with a mean baseline rate of 1–4 spikes/sec. Action potentials were characterized by either a mono- or biphasic waveform of 1.5–2.0 msec in duration. Neurons that histological analysis revealed to be outside the CAN were not included in the analysis.

Control rats consistently responded to a d-amphetamine challenge with an inhibition of neuronal activity to below 50% of the baseline rate by the 4th or 5th injection (n=12). In rats pretreated with d-amphetamine, however, the majority of CAN neurons (5 of 9) failed to respond even by the 10th

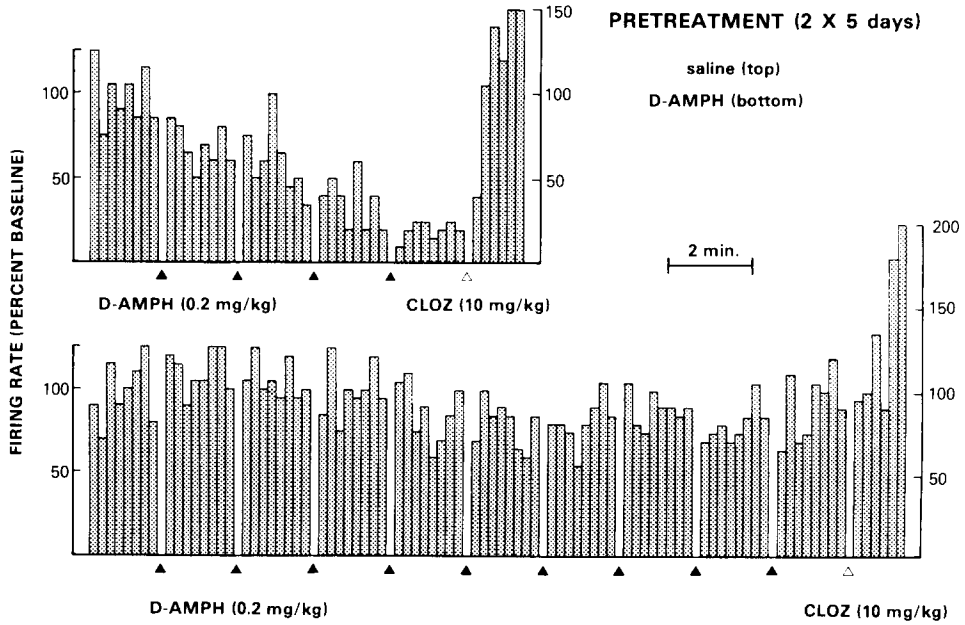

FIG. 2. Representative examples of the response to challenge injections of 0.2 mg/kg d-amphetamine in a control (top) and in a d-amphetamine-pretreated (bottom) rat. Neuronal activity was summed for 15-sec intervals and plotted as a percentage of the baseline rate which was defined at 100%. Note the lack of responsiveness in the amphetamine-pretreated animal. In both cases, however, a subsequent IV injection of clozapine (CLOZ) produced a marked increase in firing rate.

injection. Figure 1 illustrates this difference for both groups of rats. Note the dramatic shift to the right of the amphetamine response curve in rats pretreated with this drug.

The response of representative neurons in each group is shown in Fig. 2. In the saline pretreated animal, firing rate is almost completely suppressed by the 4th injection, whereas neuronal activity fails to change in the rat pretreated with d-amphetamine. Note also that a subsequent injection of clozapine increased firing rate in both groups arguing against the possibility that CAN neurons are simply unresponsive following long-term amphetamine administration.

Amphetamine pretreatment also reduced the neuronal response to apomorphine although the change was less dramatic than with the d-amphetamine challenge. As shown in Fig. 3, apomorphine inhibited the activity of 67% (6 of 9) of CAN neurons in control rats but only 22% (3 of 14) in rats pretreated with d-amphetamine. In fact, maximum inhibition in these rats occurred with a challenge dose of 0.16 mg/kg; further increases in the apomorphine dose failed to inhibit any additional neurons.

Representative examples of the apomorphine response are shown in Fig. 4. Note that in the control rat neuronal activity was suppressed to below 50% of the baseline rate by 0.64 mg/kg and this response was reversed by clozapine. Following amphetamine pretreatment, a depression failed to occur even as the apomorphine dose was increased to 1.28 mg/kg and, finally, to 2.56 mg/kg. A subsequent injection of clozapine, however, accelerated neuronal activity.

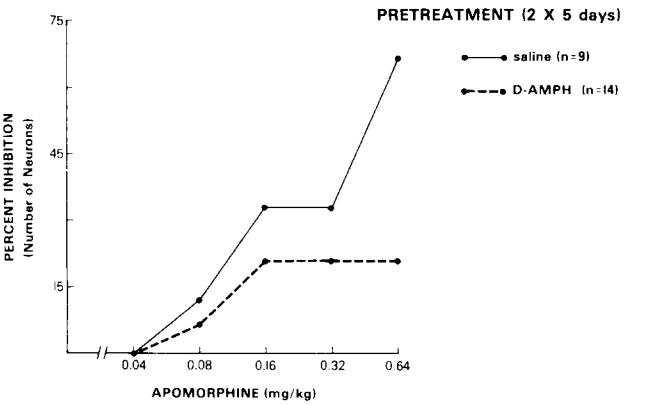

FIG. 3. Percentage of CAN neurons inhibited by IV challenge injections of apomorphine in rats pretreated as in Fig. 1. Whereas 6 of 9 control neurons were inhibited by increasing incremental doses of apomorphine, only 3 of 14 neurons were suppressed by this drug following amphetamine pretreatment.

DISCUSSION

Our results clearly indicate that tolerance develops to the inhibitory action of d-amphetamine on neurons in the CAN. This effect is in dramatic contrast to that reported for neostriatal neurons which appear to increase their responsiveness to amphetamine following long-term treatment [1, 20, 31]. Moreover, unlike neostriatal neurons, cells in the CAN also become less responsive to apomorphine. Thus, to the extent that apomorphine inhibits CAN neurons by acting directly on inhibitory DA receptors, the tolerance that de-

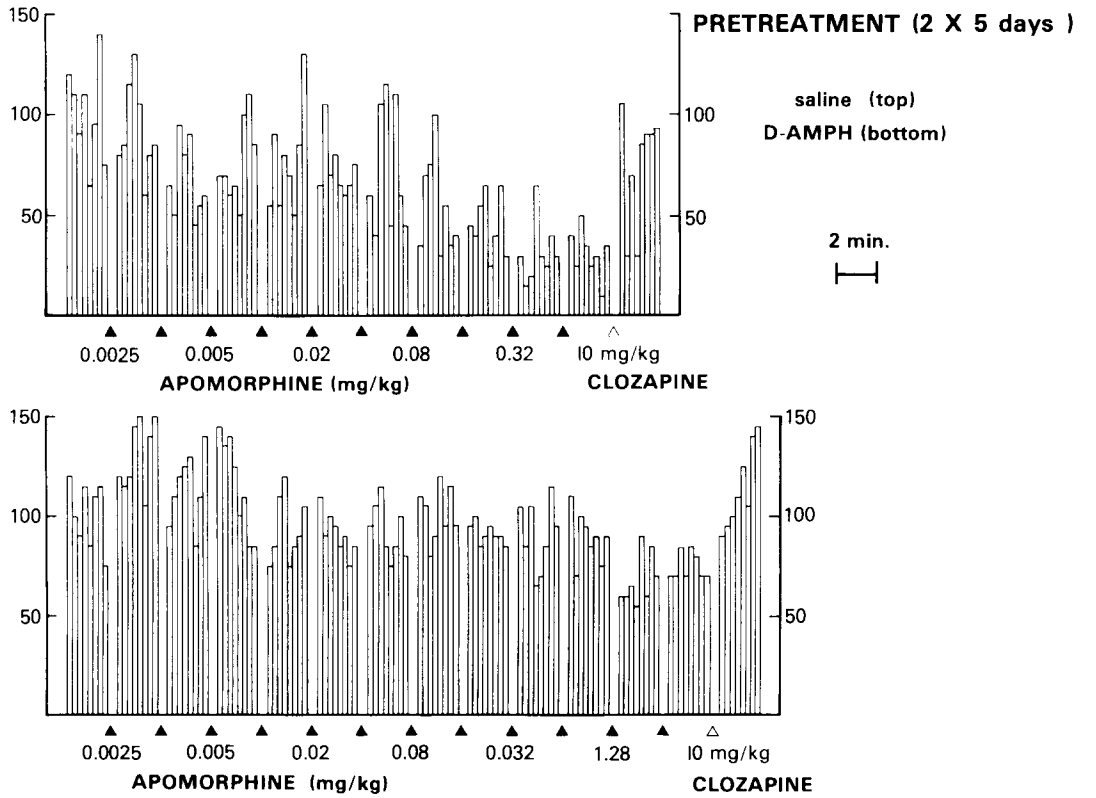

FIG. 4. Response of individual neurons in the CAN to apomorphine following saline (top) or d-amphetamine (bottom) pretreatment. Firing rate is plotted as in Fig. 2. Note again the failure to depress firing rate in the amphetamine-pretreated rat.

velops to amphetamine may reflect, in part, a decrease in postsynaptic DA receptor sensitivity. Other mechanisms also appear to be involved, however, because even in control rats a substantial portion of CAN neurons failed to respond to apomorphine. It appears, therefore, that even though the CAN receives DA input, neurons in this site are not uniformly responsive to direct acting DA agonists.

Interestingly, however, all the CAN neurons in our control sample were inhibited by d-amphetamine perhaps because, apart from facilitating DA release, this drug also increases the release of norepinephrine and serotonin [6, 13, 17, 25], both of which have been identified in the amygdaloid complex [3, 11, 12, 18]. Thus, we cannot rule out the possibility that with long-term treatment a change in a non-DA system may account for the decreased responsiveness of CAN neurons to this drug.

Both the neostriatum and the CAN receive input from DA neurons in the substantia nigra pars compacta [12, 23, 38]. Long-term amphetamine treatment has been reported to reduce the number of autoreceptors on these cells [24] which should produce less inhibition of DA neurons and a greater postsynaptic effect [27]. In fact, recordings from the substantia nigra pars compacta ([2,20] but see also [36]) and the neostriatum [1,31] support this view. The development of tolerance in the CAN, however, suggests that not all postsynaptic sites are similarly affected by amphetamine.

It is also interesting to note that in those cases in which we injected clozapine, neuronal activity rapidly increased. These results confirm previous evidence that CAN neurons are extremely sensitive to this antipsychotic drug and that clozapine can reverse the depression of firing rate produced by an intraperitoneal injection of d-amphetamine [28]. In the present study, long-term amphetamine treatment did not appear to alter the clozapine response although a more systematic analysis would be required to verify this point since the pre-clozapine firing rate was quite different in saline and amphetamine-pretreated animals. Moreover, clozapine has adrenolytic and antiserotonergic actions in addition to its DA receptor blocking properties (see [28]) making it difficult to speculate on the mechanism by which this drug reversed the amphetamine response. Nevertheless, our results demonstrate that even though CAN neurons may be completely unresponsive to amphetamine with long-term treatment, these same cells continue to respond to clozapine.

An accumulating body of evidence suggests that the CAN plays at least a modulatory role in the behavioral response to amphetamine. A variety of lesions of this site, for example, have been reported to attenuate amphetamine-induced licking and biting [5, 7, 8]. The amygdaloid complex has also been implicated in eating behavior [4, 16, 26] and arterial blood pressure [9, 14, 37], both of which are altered by amphetamine. In view of evidence that the anorexigenic and sympathomimetic effects of amphetamine as well as the oral stereotyped behavior produced by the drug show tolerance with repeated injections, it is tempting to speculate that the reduced responsiveness of CAN neurons may, at least in part, be responsible for this effect. Further research is required to examine the role of the amygdaloid complex in the tolerance that develops to specific amphetamine-induced behaviors.

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