Short communications

Effect of 6-hydroxydopamine on electrical self stimulation of the brain

G. R. BREESE, J. L. HOWARD AND J. P. LEAHY

Departments of Psychiatry, Pharmacology and Psychology and The Child Development Institute, University of North Carolina, School of Medicine, Chapel Hill, North Carolina, USA

In rats, after a single intracisternal injection of 6-hydroxydopamine (6-OHDA) electrical self stimulation was reduced by approximately 50%. The concentrations of noradrenaline and dopamine in the brain were reduced by 83%. A second injection of 6-OHDA reduced the concentration of these amines to 7% of control values and virtually eliminated self stimulation.

Several lines of evidence indicate that noradrenaline (NA) plays a crucial role in electrical self stimulation of the brain (Poschel & Ninteman, 1963, 1966; Wise & Stein, 1969). These have included the release of [^{3}H]-NA following self stimulation in the medial forebrain bundle (MFB) (Stein & Wise, 1969), the restoration of bar pressing by intraventricular injection of NA following the blockade of NA formation by disulfiram (Wise & Stein, 1969) and also an increased synthesis of NA from [^{14}C]-tyrosine (Wise & Stein, 1970). In spite of this strong support, the role of NA in self stimulation has recently been challenged by Roll (1970), who found that if the behavioural depression caused by disulfiram was allowed for, the animals bar pressed normally. Our study attempted to reassess the importance of NA in self stimulation by using 6-hydroxydopamine (6-OHDA) to reduce central catecholamine concentrations. This reduction of brain NA and dopamine (DA) (Breese & Traylor, 1970; Uretsky & Iversen, 1970) is presumed to be due to the destruction of central catecholamine nerve terminals (Bloom, Algeri, Groppetti, Revuelta & Costa, 1969; Breese & Traylor, 1970). This compound offers the distinct advantage of disappearing from the brain after producing its destructive effect and having no effect on peripheral adrenergic fibres if administered intracisternally (Breese & Traylor, 1970).

Methods.—Male Sprague-Dawley rats (350–400 g) were implanted with bipolar stainless steel electrodes (tip–0·2 mm) aimed at the MFB either at the level of the posterior hypothalamus (A–P, +3·8, M–L, −1·2, D–V−3·0) or the lateral hypothalamus (A–P, +5·8, M–L, −1·7, D–V, −2·0) (De Groot, 1959). After allowing 7 days for recovery, the animals were trained to press a bar which delivered a 120 ms, 60 Hz, 40 μA sine wave stimulus. Pressing rates varied from 1,200–4,500 per 30 minutes. Animals were tested daily for 30 min until pressing rates stabilized and then values for 5 additional days were recorded as a baseline. The animals which were assigned to the 6–OHDA and control groups were comparable with respect to electrode location and bar pressing rate. Following the fifth baseline day the experimental animals each received an intracisternal injection of 200 μg of 6–OHDA dissolved in saline acidified with ascorbic acid, while the control animals received only the vehicle. An intraperitoneal injection of pargyline (50 mg/kg) preceded the central injections by 30 min (Breese & Traylor, 1970). Thirty-five days after the first injection, 6–OHDA treated rats were given a second injection of 6–OHDA (200 ug) without pargyline. Self stimulation rates were recorded on all animals after the first injection for 48 days, including 13 days after the second injection. Self stimulation rates following the injection were expressed as a percentage of the baseline value for each animal.

At the end of the experiment, the brains of four animals from each group were assayed for catecholamine concentrations. The brains of other animals which received only a single injection of 6–OHDA were also analysed. The brain from each animal was homogenized in 10 ml of ice-cold 0·4 N perchloric acid. The homogenate was then centrifuged and the catecholamines in the supernatant were adsorbed on to alumina at pH 8·6 using a modification of the method of Anton & Sayre (1962). NA in the acetic acid eluate was assayed by the method of Häggendal (1963) and DA was assayed by the method of Anton & Sayre (1964). Values for endogenous amines were uncorrected for recovery which averaged 86% for NA and 82% for DA.

Results.—The difference between electrode placements within experimental conditions were evaluated with a Mann-Whitney-U test. Since the values for the lateral and posterior hypothalamus placements were not significantly different, the results obtained with the two placements were combined. The resulting group curves are presented in Fig. 1. The slight rise in response rate after the injection of saline to pargyline pretreated animals was not significantly different from the baseline level. After the intracisternal injection of 6–OHDA, self stimulation rates dropped to 20% of control on the day immediately after the injection, but by the second day they had recovered to approximately 50% of the baseline values. The self stimulation rates remained depressed for at least 35 days and remained in the range of 50–60% of control period rates. The difference between the saline injected and 6–OHDA injected animals was significant ($f=9{\cdot}4$; $P<0{\cdot}05$). There was no significant difference between sessions nor was there a session-group interaction.

A second injection of 6–OHDA virtually eliminated self stimulation in five of the six experimental animals. The usual result during the 15 days after the second injection was one or two bar presses per day per animal. The sixth animal showed some reduction in self stimulation rate from that observed after one injection, but the rate (37 ± 3% of control) was above the average rate for the other five (0% of control). A possible explanation for this result may be that the brain of this animal contained a higher NA concentration (24% of control) than that of the other five (7 ± 1% of control). DA concentrations in the brain were reduced by approximately 92% after the two injections of 6–OHDA. A single injection of 6–OHDA in combination with pargyline reduced the concentrations of NA and DA by 83·1±5·6 and 83·5± 6·8%, respectively.

Discussion.—The reduction of the self stimulatory rate following destruction of the catecholamine containing nerves in the brain with 6–OHDA lends support to the hypothesis that self stimulation is dependent upon a central noradrenergic system (Wise & Stein, 1969). Since the first injection which lowered the concentrations of the catecholamines in the brain by some 83% reduced self stimulation rates by 50%, the elimination of self stimulation after the second injection of 6–OHDA was unexpected in that amines were only moderately further reduced after the additional dose. Whether this slight reduction of amines is effective because the number of fibres re-

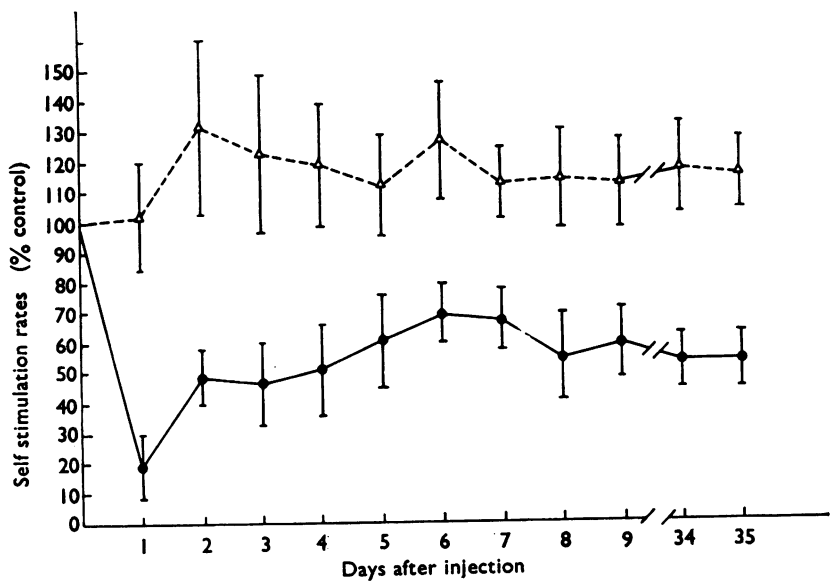

FIG. 1. Self-stimulation rates for six 6-OHDA(●——●) and five saline (△- - -△) injected animals expressed as percentage of the mean obtained during a 5 day baseline period. Vertical bars indicate standard error of the mean.

maining is at a critical level for maintaining function or whether a more complex hypothesis is required should be critically examined.

Roll (1970) has suggested that the disulfiram may have blocked self stimulation by causing a behavioural depression, thus questioning the view that the reward value of self stimulation is maintained by noradrenergic neurones. Since 6-OHDA has little or no effect on locomotor activity in chronically treated animals (Evetts, Uretsky, Iversen & Iversen, 1970; Burkard, Jalfre & Blum, 1969), behavioural depression can probably be ruled out in explaining the reduction of self stimulation rate by 6-OHDA. Furthermore, 6-OHDA has no effect on the concentration of 5-hydroxytryptamine in the brain (Breese & Traylor, 1970) or on cerebral γ-aminobutyric acid (Uretsky & Iversen, 1970) suggesting that these substances are not concerned in the reduction of the self stimulation rate by 6-OHDA.

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REFERENCES

ANTON, A. H. & SAYRE, D. F. (1962). A study of the factors affecting the aluminium oxide-trihydroxyindole procedure for the analysis of catecholamines. *J. Pharmac. exp. Ther.*, **138**, 360–375.

ANTON, A. H. & SAYRE, D. F. (1964). The distribution of dopamine and dopa in various animals and a method for their determination in diverse biological material. *J. Pharmac. exp. Ther.*, **145**, 326–336.

BLOOM, F. E., ALGERI, S., GROPPETTI, A., REVUELTA, A. & COSTA, E. (1969). Lesions of central norepinephrine terminals with 6-OH-dopamine: biochemistry and fine structure. *Science, N.Y.*, **166**, 1284–1286.

BREESE, G. R. & TRAYLOR, T. D. (1970). Effect of 6-hydroxydopamine on brain norepinephrine: evidence for selective degeneration of catecholamine neurons. *J. Pharmac. exp. Ther.*, **174**, 413–420.

BURKARD, W. P., JALFRE, M. & BLUM, J. (1969). Effect of 6-hydroxydopamine on behaviour and cerebral amine content in rats. *Experientia*, **25**, 1295–1296.

DEGROOT, J. (1959). The rat forebrain in stereotaxic coordinates. *Verho. ned. Akad. Wet.*, Sect. II, 52.

EVETTS, K. D., URETSKY, N. J., IVERSEN, L. L. & IVERSEN, S. D. (1970). Effects of 6-hydroxydopamine on CNS catecholamines, spontaneous motor activity, and amphetamine induced hyperactivity in rats. *Nature, Lond.*, **225**, 961–962.

HÄGGENDAL, J. (1963). An improved method for the fluorometric determination of small amounts of adrenaline and noradrenaline in plasma and tissues. *Acta physiol. scand.*, **59**, 242–254.

POSCHEL, B. P. H. & NINTEMAN, F. W. (1963). Norepinephrine: a possible excitatory neurohormone of the reward system. *Life Sci.*, **1**, 782–788.

POSCHEL, B. P. H. & NINTEMAN, F. W. (1966). Hypothalamic self-stimulation: its suppression by blockade of norepinephrine biosynthesis and its reinstatement by methamphetamine. *Life Sci.*, **5**, 11–16.

ROLL, S. K. (1970). Intracranial self-stimulation and wakefulness: effect of manipulating ambient brain catecholamines. *Science N.Y.*, **168**, 1370–1372.

STEIN, L. & WISE, C. D. (1969). Release of norepinephrine from hypothalamus and amygdala by rewarding medial forebrain bundle stimulation and amphetamine. *J. comp. physiol. Psychol.*, **67**, 189–198.

URETSKY, N. J. & IVERSEN, L. L. (1970). Effects of 6-hydroxydopamine on catecholamine-containing neurons in the rat brain. *J. Neurochem.*, **17**, 269–278.

WISE, C. D. & STEIN, L. (1969). Facilitation of brain self-stimulation by central administration of norepinephrine. *Science, N.Y.*, **163**, 299–301.

WISE, C. D. & STEIN, L. (1970). Increased biosynthesis and utilization of norepinephrine during self-stimulation of the brain. *Fedn Proc.*, **29**, 485.

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