Clonidine-induced body temperature changes in rats with anterior or posterior cortical damage

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Animals with anterior or posterior neocortical lesions as well as intact control animals were evaluated for changes in α -adrenergic sensitivity at five postoperative times. Colonic temperature decreases were measured in response to clonidine at each of the postoperative periods. The result indicated that a heightened heat-loss response was induced by clonidine that lasted 21 or more days after destruction of the posterior neocortex. This exaggerated response to the drug was not found in animals with anterior neocortical lesions.

In studying the induction of supersensitivity of the norepinephrine (NE) mediated systems, Zis and Fibiger (1975) found that the reduction in colonic temperature produced by clonidine was substantially increased by prior injection of 6-hydroxydopamine into ascending NE systems in the brainstem. Their evidence suggested that destruction of the ventral NE bundle was critical to this effect, which was found both 1 and 3 months postoperatively. These lesions greatly reduced hypothalamic NE levels but did not affect NE levels in cortex or hippocampus.

The effects of central application of NE in regard to body temperature seem to be the result of its α -adrenergic actions in many species, including the mouse (Brittain & Handley, 1967), cats (Burks, 1972), and rabbits (Dhawan & Dua, 1971). Further support for the importance of α -adrenergic stimulation on the reduction of body temperature comes from studies in which the temperature loss produced by intraventricular administration of NE could be blocked by α - but not by β -antagonists (Cantor & Satinoff, 1976).

Clonidine is considered to be a stimulator of α -adrenergic receptors (Anden, Corrodi, Fuxe, Hokfelt, Hokfelt, Rydin, & Svensson, 1970). Supporting this view, it acts in a negative feedback fashion to regulate the release of noradrenaline from both peripheral and central neurons (Cubeddu, Barnes, Langer, & Weiner, 1974; Haggendal, 1973; Hedqvist, 1974; Kirpekar, Furchgott, Wakade, & Prat, 1973; Starke & Montel, 1973, 1974; Stjarne, 1975). Administration of the drug by intracranial or intraperitoneal routes reduces colonic temperature (Taylor & Laverty, 1969; Tsoucaris-Kupfer & Schmitt, 1972). The hypo-

thermic response to clonidine administration can be eliminated by treatment with phenoxybenzamine, and α -adrenergic antagonist (Reid, Lewis, & Meyers, 1975) but not by propranolol, a β -adrenergic antagonist (Fukushima & Itoh, 1975).

The effect of the α -adrenergic agonist clonidine is independent of ambient temperature, producing hypothermic responses of similar magnitude at both 6° and 20°C.

Several forms of brain damage have been shown to produce progressive alterations in behavior and in reactions to pharmacologic agents. This has been shown after damage to the hippocampus (Lanier & Isaacson, 1975; Lanier, Petit, & Isaacson, 1974) and other brain areas (e.g., Campbell, Ballantine, & Lynch, 1971; Glick & Marsanico, 1974; Miczek & Grossman, 1972; Miczek, Kelsey, & Grossman, 1972; Pompei, 1974).

Neocortical lesions produce differences in the incorporation of [³H] uridine at long and short postoperative periods (Wells, 1976), and other progressive changes in behavior after neocortical damage can be found in other studies (e.g., Lanier et al., 1974). Furthermore, differential sensitivity to receptor stimulating drugs can be found after lesions in different cortical regions of the rat neocortical surface. For example, Glick and Cox (1976) found enhanced sensitivity to apomorphine after frontal lesions, but not to clonidine. The frontal cortex of the rat seems to receive a relatively dense DA input, especially to the deeper cortical layers (Bunney & Agahajanian, 1976). In general, there is a widespread distribution of NE innervation of the rat neocortex (Dahlstrom & Fuxe, 1964; Ungerstedt, 1971), although there may be a preferential distribution of NE from locus coeruleus to the posterior neocortex. There is a greater decrease of NE in posterior than in anterior areas after lesions of the locus coeruleus (Worth, Collins, Kett, & Austin, 1976).

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The present research represents an initial effort to determine the time course of possible differential adrenergic responsivity of rats to lesions of the anterior and posterior neocortical surface. The reduction of colonic temperature by clonidine was used as the measure of responsiveness. This adrenergic agonist was chosen because of its selective activation of α -receptors and to make the study comparable to that of Zis and Fibiger (1975), who studied changed adrenergic sensitivity induced by other methods with this drug.

METHOD

The subjects were male hooded rats of the Long-Evans strain, housed in individual cages. The room was maintained on a 12-h/12-h light-dark schedule. Food and water was available ad lib.

All operated animals were anesthetized with pentobarbital. Under clean surgical procedures, the scalp was opened and holes trephined over anterior or posterior neocortical regions. The dura was opened and small lesions were made by aspiration under visual guidance. In most animals, the skull openings and lesions were made bilaterally, although three animals received unilateral lesions. After surgery, the scalp was replaced and sutured. The animals received 60,000 units of Bicillin postoperatively. On completion of the experiment, the animals were sacrificed with an overdose of pentobarbital, intracardially perfused with saline followed by Formalin, and the brains removed. Photographs of the dorsal surface were made before processing for histological evaluation. The brains were embedded in celloidin, sectioned, mounted, and stained with thionin for histological evaluation. All animals with signs of superficial meningeal infection or incidental damage to subcortical structures were discarded from the data analysis.

The data from five animals with posterior neocortical damage, four animals with anterior neocortical damage, and seven control animals, are reported. Three of the seven control animals received unilateral neocortical damage but were not behaviorally different from the intact animals and were combined with the results from four other intact control animals. Composite drawings of the bilateral cortical lesions are shown in Figure 1.

The devices used to restrain the animals were constructed of cylindrical Plexiglas approximately 20.5 cm in length and 7 cm in diameter. Each cylinder was longitudinally sliced and glued to a flat Plexiglas bottom of 6 cm wide. Each cylinder was enclosed at one end by a Plexiglas strip. Several holes were drilled in these strips to allow for ventilation. The opposite ends of the chambers were closed by means of horseshoe-shaped gates which could be adjusted to the length of the animal. A slot in the gate allowed the experimenter to manipulate the rat's tail, which facilitated insertion of the thermometer probe.

All testing was done in the same experimental room at an ambient temperature of 22°-23°C. The rats were allowed to rest in the restraining devices for 10 min before the initial temperature recording. Rectal temperature was recorded by inserting the rectal probe of a Yellow Springs Instrument Company Model 73 Tele-Thermometer, 5 cm into the rectum. The probe was cleaned after each use with alcohol and coated with petroleum jelly before insertion. Four days before surgery, the animals were restrained and injected with 1.0 ml/kg physiological saline. Ten minutes after being placed in the restraining chamber, their mean initial temperatures were 37.83°C. The injection of saline produced an increase of 0.18°C (SEM = .06) 75 min afterwards. The next day, 3 days before surgery, the animals were injected with 0.1 mg/kg clonidine, following the same procedure. This produced a mean decrease of 2.62°C (SEM = 0.17) in the temperature of the animals. The postinjection time of the maximal decrease was

Figure 1. Drawings of lesions of the neocortical damage in the anterior (left) and posterior lesion groups. Drawings made on the basis of photographic analysis of brain surfaces and subsequent histological confirmation of lesion extent.

75 min. The decrease in temperature found before surgery after the first injection of clonidine was greater than found in control animals during postoperative testing. This may have been due to the animals' greater struggling to escape from the apparatus during the initial testing sessions, or it may be due to a lasting change in responsiveness to clonidine following this first administration.

In testing after surgery, 10 min after an initial temperature recording in the apparatus, each animal was injected IP with 0.10 mg/kg clonidine through a slot in the floor of the chamber. Subsequent temperature recordings began 15 min after the injection, and were repeated at 15-min intervals for a duration of 120 min. This procedure was repeated for all groups at 4, 7, 14, 21, and 28 days after surgery.

RESULTS

The postoperative baseline temperatures recorded before injection did not differ among the groups or differ from those recorded in the same manner 3 days preoperatively. The extremes of temperatures recorded before the drug was given on the 4th postoperative day ranged from 37.37° to 38.0°C. No systematic changes in baseline occurred over the 5 postoperative test days in any of the groups.

The data were analyzed on the basis of decreases in colonic temperature produced by the clonidine for each animal. The median maximal decreases for each group are shown in Figure 2.

The times after injection at which the greatest temperature reductions occurred became longer after the injection for all groups except the animals with anterior neocortical damage. For example, on Postoperative Day 4, the median maximal decrease occurred at 1 h for both animals with posterior cortical damage and the control subjects. On Postoperative Day 28, the maximal decreases occurred 1.5 h after injection for both groups. Evaluation of these changes by a sign test showed the changes to be significant ($p < .025$). The anterior lesion group, on the other hand, showed a delayed maximal response to clonidine (median: 1.5 h) which remained the same through the course of testing.

To determine if changes in the maximal tempera-

Figure 2. Mean maximal temperature decreases after clonidine for animals with anterior and posterior neocortical lesions and control animals. Vertical bars at each mean indicate SE of group scores.

ture decreases occurred across testing periods, Friedman analyses were made for all groups. The only group for which a significant change across postoperative periods was found was the group with anterior cortical lesions ($p < .05$). As can be seen in Figure 2, this was due to a depression in the clonidine-induced reaction when tested 4 days after surgery. A Friedman test made on this group for the last four postoperative periods was not significant. All animals in this group had temperature reductions comparable to those of the control group on Postoperative Days 7-28.

To evaluate the enhanced temperature drop found in the animals with posterior cortical lesions, comparisons were made between the posterior lesion and control groups. Since the Friedman test failed to show changes over testing days for these groups, a Mann-Whitney U test was performed between the mean decreases from all testing days for the two groups. The results indicated a significant decrease in temperature for the posterior lesion group relative to the control subjects ($p < .01$).

From the data presented in Figure 2, it appears that there is a convergence in the mean temperature decreases between the posterior lesion group and the controls on Postoperative Day 28, but with the

absence of a significant change, as indicated by the Friedman test, the conclusion of a convergence must be considered tentatively.

DISCUSSION

The data obtained in the present study indicate an enhanced sensitivity to clonidine, as measured by a decrease in body temperature after the drug, which lasts at least 3 weeks after surgery and possibly longer. The present data do not allow a firm conclusion that the hypersensitivity found in the posterior lesion group has dissipated by 28 days. The group of animals that received anterior lesions did *not* show an enhanced reaction to clonidine and, indeed, were hyposensitive to the drug 4 days after surgery. On subsequent postoperative testing days, they were indistinguishable from control animals. Further, unilateral lesions of the neocortical surface failed to affect clonidine sensitivity.

Following the interpretation of Zis and Fibiger (1975) of the enhanced responsiveness to clonidine, that is, the induction of supersensitivity in central α -adrenergic neurons, our data would suggest that posterior neocortical lesions induce a supersensitivity of NE-sensitive neurons, as well. However, such a conclusion must be tempered by the possibility that the clonidine-induced reaction may be a consequence of changes in other neurotransmitter systems. Clonidine could have produced its effects by stimulation of epinephrine (E) receptors (Bolme, Corrodi, Hokfelt, Lidbrink, & Goldstein, 1974), changing ACTH or corticosteroid secretion (Ganong, Kramer, Salmon, Reid, Lovinger, Scapagnini, Boryczka, & Schackelford, 1976; Van Loon, Scapagnini, Cohen, & Ganong, 1971), by activating histamine H₂-receptors (Sastri & Phyllis, 1977), or by indirect effects on serotonergic (5-HT) systems (Svensson, Bunney, & Aghajanian, 1975).

The possible return of normal colonic temperature responses to clonidine in the lesioned rats could reflect a plasticity in the catecholamine systems. Both collateral and "compensatory" sprouting have been demonstrated in these systems, at least when neurochemical lesions are made early in life (Devor & Schneider, 1975; Jonsson, 1976; Jonsson, Pycck, & Sachs, 1973; Sachs & Jonsson, 1975; Schneider & Jhaveri, 1974). After early administration of 6-hydroxydopa, a permanent decrease in forebrain NE is found, but NE increases in the brainstem are also found. This is thought to be due to the accumulation of NE in anomalous axon sprouting in the brainstem (Zieher & Jaim-Etcheverry, 1973). However, these sprouting changes are not limited to the damage in the infant brain (see Moore, 1975, for review).

The effect of clonidine on DA systems is unlikely to be of importance for the present study, because these effects are found with low ambient tempera-

tures and not at those used in the present study (Reid et al., 1975; Yehuda & Wurtman, 1972). However, changes in the DA systems have been reported that may be related to the short-term effects found after frontal cortical damage, an area receiving considerable DA innervation.

The ability of DA-sensitive neurons to develop supersensitivity has been shown in a number of ways. These include denervation (Ungerstedt, 1971) and the administration of agents that block or reduce DA transmission (Gianutsos, Drawbaugh, Hynes, & Lal, 1974; Tarsy and Baldessarini, 1973; Voigtlander, Losey, & Triezenberg, 1975; Yarbrough, 1975).

In all likelihood, however, the effect of clonidine on body temperature found in this study is due to its α -adrenergic receptor stimulating effects. These involve a decrease in body temperature by means of an enhanced heat-loss mechanism (Fukushima & Itoh, 1975). Nevertheless, as mentioned above, changes in other neural mechanisms cannot be excluded as causes of the changes found in this study.

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