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BRIEF COMMUNICATION

Effect of Hypophysectomy on Schedule-Induced Wheelrunning

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LIN, W., G. SINGER AND D. IRBY. *Effect of hypophysectomy on schedule-induced wheelrunning.* PHARMACOL BIOCHEM BEHAV **35**(3) 739–742, 1990.—Previous research has shown that adrenalectomy suppresses schedule-induced wheelrunning, and that the suppressant effect of adrenalectomy can be totally reversed by the replacement of corticosterone. The present study confirms the role of the hormones of the pituitary adrenocortical axis in the control of schedule-induced wheelrunning by means of hypophysectomy. As anticipated, hypophysectomy also suppressed schedule-induced wheelrunning. The suppressant effect of hypophysectomy on schedule-induced wheelrunning was partially, but significantly restored by the implantation of corticosterone. Present findings, in conjunction with the previous work, show that schedule-induced wheelrunning is markedly dependent on the function of the pituitary-adrenal axis, and the nature of pituitary-adrenal involvement is mainly through circulating corticosterone levels.

Schedule-induced wheelrunning Pituitary-adrenal Rats Hypophysectomy Adrenalectomy ACTH Corticosterone

WE have recently observed that adrenalectomy abolishes schedule-induced wheelrunning (SIW) and that the suppressant effect of adrenalectomy can be reversed by replacement of corticosterone, suggesting that adrenal corticosterone plays an important role in SIW (12). Given a pituitary-adrenal feedback system any changes in adrenocortical activity are accompanied by changes in the level of adrenocorticotropic hormone (ACTH) secreted by the pituitary (1,11). Bilateral adrenalectomy leads to a sustained increase in ACTH levels (4, 6, 9, 13, 18), while treatment with exogenous glucocorticoids leads to a decrease in ACTH levels (8,22). Many behavioural changes which involve glucocorticoid changes appear to be consequences of pituitary action (2, 3, 5, 15, 19). Therefore, it can be argued that the suppression of SIW following adrenalectomy and the reversing effect of corticosterone are a result of changes in circulating ACTH levels which follow these manipulations, rather than a direct consequence of corticosterone levels per se.

In order to clarify the role of the hormones of the pituitary adrenocortical axis in the control of schedule-induced behaviour, the first experiment was designed to examine the effect of hypophysectomy, which leads to both low corticosterone and low ACTH levels (7,17), on SIW. The second experiment was to investigate the effect of exogenous corticosterone on SIW in hypophysectomized rats. In addition, the issue of whether adrenal medullary secretions are involved in the regulation of schedule-induced behaviour, raised previously (12), will also be discussed.

To monitor the function of the pituitary adrenocortex system under various conditions, plasma corticosterone levels of all experimental rats were measured at the termination of the experiment. Changes in the weight of adrenal glands were also observed.

METHOD

Subjects

Thirty male hooded Long-Evans rats with an initial body weight of about 300 g and aged between 90 and 120 days were used. Rats were housed individually under temperature-controlled conditions (22 ± 1°C) with a 12-hour light/12-hour dark cycle. After a period of acclimatization to the laboratory, the rats were body-weight reduced, over a fourteen-day period of restricted food intake, to 80% of their free-feeding body weight, and were

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maintained at this weight throughout the experiment, unless otherwise stated. Water was freely available at all times.

Apparatus

The test chamber was made of clear perspex with a stainless steel barred floor, and measured 33.5 × 28 × 42 cm. A food cup was located on one end wall of the chamber approximately 3.5 cm above the floor. Pellet delivery was automatically controlled by standard relay circuitry. Noyes standard formula 45 mg food pellets were used. A 26 cm diameter running wheel was at the rear of the chamber and turned in either a clockwise or counter-clockwise direction. The number of wheel revolutions was recorded on a five-digit electromechanical counter. Experimental sessions were conducted in eight chambers simultaneously. All rats were exposed to a FT 120 sec nonreinforcement contingent schedule, under which they received one 45 mg pellet each two minutes, for 1.5 hours at the same hour of day for 10 consecutive days.

Surgery

The pituitary was removed by the parapharyngeal approach under ether anaesthesia (21). Sham-operated rats underwent the same surgical procedure, but the skull was not penetrated by the drill and, thus, the pituitary was left intact. For rats in the vehicle- or corticosterone-treated hypophysectomized group a pellet consisting of 100 mg of cholesterol or 50 mg of corticosterone combined with 50 mg of cholesterol (14) was implanted subcutaneously in the nape of the neck at the completion of surgery. On the day following surgery all of the operated rats were given an IP injection of 7.5 mg of hydrocortisone and 18,000 units of procaine penicillin IM. Hypophysectomized rats were kept in a warm room (25–27°C) for the first 3 days after surgery (21). A 5% dextrose in 0.9% NaCl solution was provided for the first 6 days following surgery (21), then 0.9% NaCl solution was available for the remaining days.

Procedure

Sixteen rats were used in the first experiment. Eight of them underwent hypophysectomy surgery (Hypox), while the other eight served as sham hypophysectomy controls (Sham). Following hypophysectomy or sham hypophysectomy rats were allowed a 10-day postoperative recovery period prior to testing. During this time, 80% body weight was reestablished.

In the second experiment, fourteen rats were hypophysectomized. Seven of them received corticosterone pellets [Hypox(C)] and the remainder, of which one died, were treated with the cholesterol pellets as vehicle implantation [Hypox(O)]. In a pilot experiment, it was found that corticosterone implantation only partially reversed SIW in hypophysectomized rats which were reduced to 80% of their initial free-feeding body weight. To avoid the possibility that the partial reversion might be, or in part, due to a possible reduction in the drive aspect with respect to the schedule feeding resulting from the surgery, all hypophysectomized animals with or without corticosterone implantation were controlled to reduce body weight individually to about 70% of their initial free-feeding weight after they reached a stable weight within 7–10 days posthypophysectomy. All operated animals, therefore, were allowed a 15-day postoperative recovery period prior to testing.

Biochemical Assay

Plasma corticosterone levels were measured using a modification of the competitive protein binding globulin assay described by Murphy (16). Horse serum provided the source of binding protein. All subjects were killed by decapitation at the completion of ten test sessions. Trunk blood was collected in heparinized tubes and centrifuged at 3200 rpm for 20 minutes. The plasma was stored at −80°C until assayed.

Measurement of Adrenal Gland

Adrenal glands were removed from the animals immediately after the collection of blood. Each gland was dissected free from periadrenal tissue and weighed on an electric balance.

RESULTS

Schedule-Induced Wheelrunning

Effect of hypophysectomy. One case, in which hypophysectomy was judged incomplete on visual inspection of remaining pituitary tissue as well as the determination value of 26.4 μg/100 ml plasma corticosterone, was discarded from all analyses. The mean number of wheel revolutions over the 10-day period for both the hypophysectomized and sham-operated groups are shown in Fig. 1a. A 2 treatments × 10 days two-way ANOVA with one repeated measure showed a highly significant treatment effect, $F(1,13) = 17.16$, $p<0.001$, day effect, $F(9,117) = 9.76$, $p<0.001$, and day × treatment interaction, $F(9,117) = 9.03$, $p<0.001$, suggesting a significant reduction in SIW in hypophysectomized animals.

Effect of corticosterone implantation. The mean number of wheel revolutions over the 10-day period for the hypophysectomized rats with and without corticosterone treatment is shown in Fig. 1b. A two-way ANOVA with one repeated measure showed a significant treatment effect, $F(1,11) = 11.53$, $p<0.01$; whereas the day effect and the day × treatment interaction were not significant. The analysis indicates that corticosterone implantation significantly increased SIW in hypophysectomized rats. However, as shown in Fig. 1b, this increase reached a plateau after the third day of testing. This result was the same as that found in the pilot study.

Pituitary Adrenocortical Function

Plasma corticosterone levels. No detectable levels of plasma corticosterone were observed in hypophysectomized or vehicle-treated hypophysectomized rats, confirming the completeness of hypophysectomy. The plasma corticosterone level in sham-operated rats was 21.6 ± 3.4 μg/100 ml, whereas the corticosterone-treated rats had a level of about 5 μg/100 ml which was below the expected level of 10–15 μg/100 ml (12,14).

Adrenal weights. A one-way ANOVA showed a significant treatment effect, $F(3,27) = 8.51$, $p<0.0005$. Post hoc Newman-Keuls tests at the 0.05 level of significance showed that mean adrenal weights for all hypophysectomized groups [Hypox, Hypox(O), Hypox(C)] were significantly lower than those of sham-operated rats. The very marked atrophy of adrenals in all hypophysectomized rats (69–71% of the controls in adrenal weight) provides additional morphological evidence of the effectiveness of hypophysectomy.

DISCUSSION

The present experiments show that hypophysectomy completely suppresses SIW, and that corticosterone implantation partially, but significantly reverses the suppressant effect of hypophysectomy on SIW. These results, combined with the earlier work which showed that SIW was suppressed by adrenalectomy,

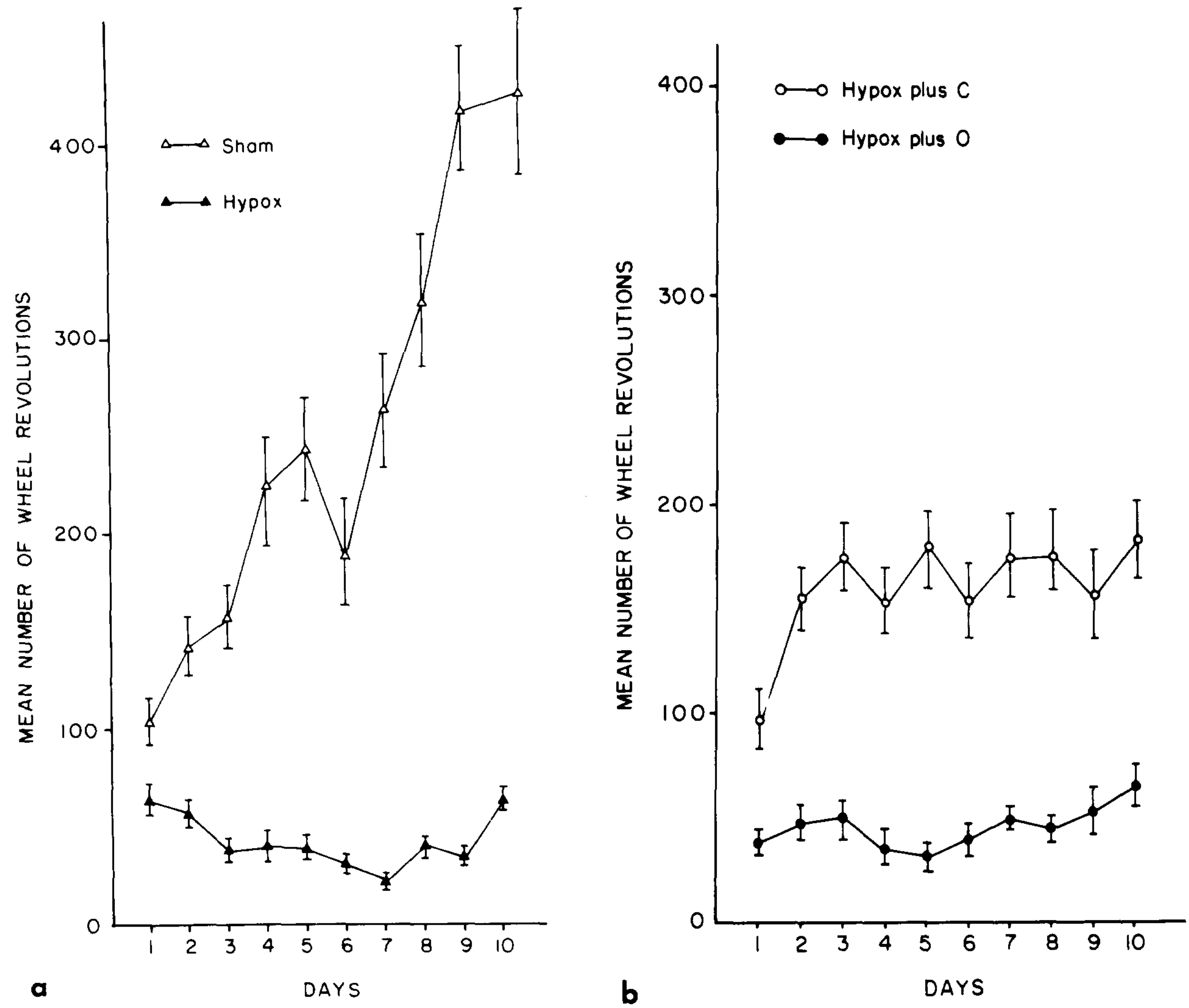

FIG. 1. (a) Wheel revolutions (Mean ± SE) during 1.5 hours of exposure to schedule food for hypophysectomized and sham-operated rats. (b) Wheel revolutions (Mean ± SE) during 1.5 hours of exposure to schedule food for hypophysectomized rats with and without corticosterone therapy.

suggest that the suppressant effect of hypophysectomy in the present experiments was not due to lowered ACTH levels, since adrenalectomy leads to an increase of ACTH levels. The results support the hypothesis that the suppressant effect of SIW following both adrenalectomy and hypophysectomy is due to the lack of circulating corticosterone levels, rather than to changes in ACTH levels. The counteractive effect of corticosterone implantation under both conditions further supports this argument.

However, corticosterone implantation did not fully restore SIW in the hypophysectomized rats as it did in the adrenalectomized rats (12). This finding can be interpreted in two different ways. 1) It is possible that although corticosterone is a necessary condition for the occurrence of SIW, it is not a sufficient condition for the full development of SIW. Hypophysectomy not only eliminates the source of ACTH and consequently glucocorticoid secretion, but also removes the source of various anterior and posterior pituitary hormones (7,17). These pituitary factors, or some of them, may have synergistic actions with corticosterone in the control of SIW. 2) The partial recovery of SIW might be due to an insufficient level of circulating corticosterone. Since the plasma corticosterone was only 5 μg/100 ml, the circulating level of corticosterone may have been at an insufficient level when compared with the earlier work on adrenalectomy (12). This suggests that the level of circulating corticosterone contributes to the determination of the animal's level of SIW. The unexpectedly low level of plasma corticosterone in this experiment raises the question of whether the metabolic degradation of exogenous corticosterone increases, or the local tissue blood flow and steroid uptake decrease in animals with the pituitary gland removed. Before definitive conclusions can be reached with regard to the role of corticosterone in SIW in hypophysectomized rats, a full dose level study needs to be carried out.

Although schedule-induced drinking has been considered to be related to adrenal medulla function (20), and some other behaviours have been reported to be identically modulated by either adrenocortical glucocorticoids or adrenomedullary secretions (10), our findings suggest that this is not the case for SIW. The fact that SIW was totally suppressed by hypophysectomy, which stops the secretions from the adrenal cortex, but not from the medulla (7,17), implies that adrenomedullary secretions do not play a significant role in SIW. Similarly, the fact that the suppressant effect of adrenalectomy on SIW was completely reversed by corticosterone replacement (12) implies that the adrenomedullary secretions are not necessary for recovery of SIW. Taken together, these findings suggest that the adrenal medulla plays no role, or no important role, in the regulation of SIW, while the pituitary-adrenal cortex is of crucial importance in the regulation of SIW.

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