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ACTH Self-Administration in Rats

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JOUHANEAU-BOWERS, M. AND J. LE MAGNEN. *ACTH self-administration in rats*. PHARMAC. BIOCHEM. BEHAV. **10**(3) 325–328, 1979.—The ability of rats to learn a lever pressing response reinforced by a contingent intravenous delivery of $ACTH_{1-24}$ and $_{4-10}$, was investigated. About 60% of the rats tested learned to press the lever for the two analogues that were infused at a rate of 2.08 μg per second via a chronically implanted intrajugular catheter. Most of the animals exhibited sustained daily self-injections of 250 to 375 μg of $ACTH_{1-24}$ and more variable amounts of $ACTH_{4-10}$. Comparisons with the self-administration of saline and altered responses following a change in the concentration of $ACTH_{1-24}$ solution indicated a positively reinforcing effect for the neuropeptide. The self-administration of the $ACTH_{4-10}$ analogue demonstrates that this reinforcing property of ACTH is not due to its adrenal stimulating activity. Brain targets possibly involved in the self-administration of ACTH are discussed.

ACTH analogues Self-administration Neuropeptides Brain rewarding system

THE POTENT behavioral effects of the pituitary adrenocorticotrophic hormone (ACTH) and of its analogues is well established [4, 5, 21]. ACTH administration restores the deficiency of avoidance learning in hypophysectomized rats [6,7]. In intact rats, the pituitary peptide retards the extinction of a learned active avoidance response [1, 2, 16, 17]. Associated with both positive and negative reinforcers, ACTH also seems to affect the learning of approach or aversive responses in a dose dependent fashion [12,14]. In addition to the effects on the acquisition and maintenance of experimentally learned behavioral sequences, it has been shown that ACTH also modifies naturally occurring ingestive and sexual behavioral patterns [3, 13, 19].

The mechanism of action of ACTH on the CNS is not yet fully understood [8,9]. It does not appear to be mediated by adrenocortical hormonal release. The $ACTH_{4-10}$ analogue which does not stimulate adrenocortical release is similarly effective. ACTH specific binding sites or receptors in the CNS have not yet been identified. The possible action of the peptide on brain opiate receptors has been suggested [10,22]. The known activity of ACTH on brain protein metabolism and on catecholamine metabolism [8] has also been suggested.

The observed behavioral effects have been interpreted as effects on short-term memory processes or on the retention of newly learned responses [12]. Since ACTH is released by stress, the effects in avoidance conditioning could also be interpreted as being a consequence of the action of ACTH on the state of alertness or defensive motivation. Another and more general model would be to consider that ACTH, injected in a training or extinction procedure, acts through its own reinforcing properties, interfering with the particular reinforcer being used.

The present study was conducted to determine if a dose dependent positive or negative reinforcing effect due to ACTH administration occurs and to evaluate the ability of rats to learn and maintain the intravenous self-administration of ACTH.

METHOD

Adult male Wistar rats, weighing 360 ± 5 g at the beginning of the experiment, were used. They were implanted with a chronic intrajugular catheter, according to the technique of Steffens [20]. The animals were placed in cylindrical home cages and were equipped for intravenous self-administration which allowed free movement despite the chronic catheter. A motor driven syringe was activated by pressing a lever which the rats had access to at all times. The syringe was inactivated upon the cessation of pressing. Each lever-press delivered the solution contained in the syringe through the catheter at a flow rate of 0.25 ml/min. A 24-hr record of lever-pressing made it possible to determine the amount and temporal pattern of self-administration. Food and water were available at all times. In some rats (N=13 rats) the feeding pattern was also recorded by a food-cup weighing device.

Experiment 1

A solution of $ACTH_{1-24}$ (Ciba-Geigy) diluted in isotonic saline at a concentration of 500 μg/ml was used. Each lever press delivered 2.08 μg of ACTH per second.

In the first group, 13 rats were initially trained during 8 days to press the lever, reinforced by the contingent delivery of the ACTH solution and then shifted to reinforcement during 5 days on a pure isotonic saline solution. In a second group, 13 rats, initially trained during 8 days on a saline solution, were shifted during the 5 subsequent days to ACTH.

Experiment 2

Twenty-three rats were initially tested during 8 days for self-administration of $ACTH_{1-24}$ solution at 500 μg/ml. They were then tested during the 5 subsequent days, with either a concentrated solution of $ACTH_{1-24}$ at 1000 μg/ml (N=10 rats, Group 1), or a diluted solution of 250 μg/ml (N=13 rats, Group 2).

Experiment 3

Using the same experimental procedure, described for the two preceding experiments, 18 rats were tested during 8 days with an $ACTH_{4-10}$ solution at 500 μg/ml (ORGANON), instead of the $ACTH_{1-24}$ analogue.

RESULTS

Experiment 1

The distribution of the mean daily amount of ACTH self-injected by the 26 rats, initially trained either with ACTH or with saline, is illustrated in Fig. 1. A pronounced bimodal distribution is conspicuous. Eleven rats, 4 belonging to the first group, and 7 to the second group, pressed on the lever delivering ACTH less than 60 sec per day; thus they obtained less than 0.25 ml of solution, corresponding to 125 μg of ACTH. The same 11 rats, contrary to the other 15 rats, also self-injected less than 0.25 ml per day of the control saline solution, offered prior to or after ACTH. Thus 11 out of 26 rats were "non-responding" in that they did not learn to press the lever, when reinforced either by ACTH or by pure saline solution. The recorded 0 to 60 sec of lever-pressing per day was a random effect.

The 9 rats of the first group that were initially trained on ACTH reinforcement which learned to press more than 60 sec per day immediately exhibited a consistent daily rate of ACTH self-injection. This contrasted with the progressive increase in lever-pressing observed for the 6 rats of the second group, initially trained on the saline solution. The daily cumulated intake of ACTH or of saline, resulted from successive short bouts of lever-pressing each lasting from 15 to 20 sec. These bouts were irregularly distributed over-time, but occurred almost exclusively during the night. In rats, whose feeding pattern was simultaneously recorded, no clear relation between the meal and lever-pressing pattern was apparent.

For the 15 rats which effectively learned to press for ACTH and also pressed for a substantial amount of saline prior to or after ACTH, the sustained amounts of ACTH obtained daily were remarkably grouped within the range of 250 to 375 μg (Fig. 1). Comparisons with the saline intake in the ACTH-saline and saline-ACTH groups demonstrate the specificity of the reinforcing effect of ACTH (Fig. 2). The 9 rats of the ACTH-saline group when shifted from ACTH to saline dramatically and significantly reduced their daily intake volume. For 6 rats of the saline-ACTH group the mean level of daily ACTH self-administration during the 8 days was within the range of 250 to 375 μg. Among these 6 rats initially trained on saline, 2 rats significantly decreased their intake volume when shifted from saline to ACTH. Therefore only 4 of the 6 rats came to obtain a daily amount of ACTH in the range of 250 to 375 μg per day (Fig. 2).

Experiment 2

Among the 23 rats initially trained on the ACTH solution

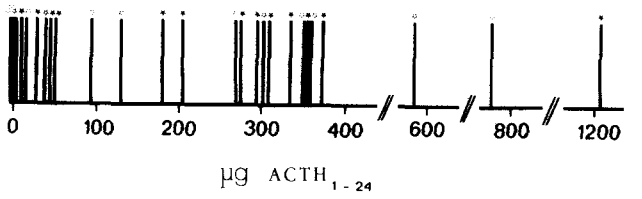

FIG. 1. Distribution of mean daily amounts of self-injected $ACTH_{1-24}$ in 26 rats. ★ Rats initially trained with ACTH reinforcement. ☆ Rats initially trained with saline reinforcement.

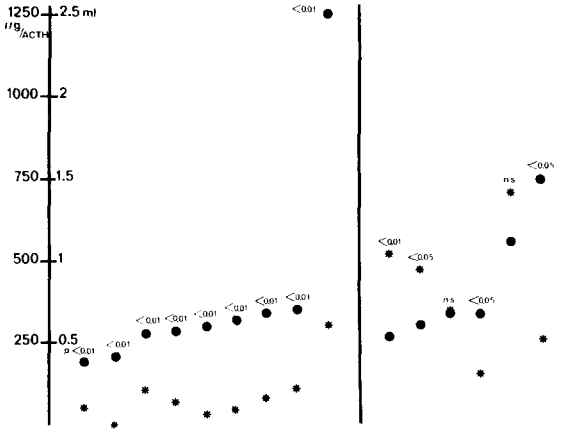

FIG. 2. Individual daily self-injection of saline (*) and of $ACTH_{1-24}$ (●) in 15 responding rats. On the left: rats initially trained with ACTH; on the right: rats initially trained with NaCl.

of 500 μg/ml, 12 rats learned the lever-pressing response. Most of them attained, as did the rats in the first experiment, a mean daily amount of 250 to 375 μg of ACTH. Among six responding rats of Group 1, 3 rats significantly reduced their daily intake volume, upon increased concentration of the ACTH solution. One rat increased, and 2 maintained their intake volume. Among six responding rats of Group 2, 2 rats significantly increased their daily intake volume upon ACTH dilution. The 4 remaining rats either decreased or maintained their daily intake (Fig. 3).

Thus, all rats but 3 altered their intake volume under the influence of changing the ACTH solution's concentration. Five responded in the compensatory direction by decreasing their intake upon increased concentration (N=3 rats), or increasing their intake upon dilution (N=2 rats). These latter animals reestablished the previous level of their daily absolute intake of ACTH. In Group 2, 2 rats suppressed their self-administration upon dilution. This loss of the response might be interpreted as a threshold effect. Eleven rats, non-responding on the initial solution, persisted in their non-response when tested on either the concentrated or the diluted solution.

Experiment 3

Among the 18 rats, 8 were non-responding, and 10 learned the self-injection of $ACTH_{4-10}$. On this analogue, they established a sustained daily self-administration pattern, com-

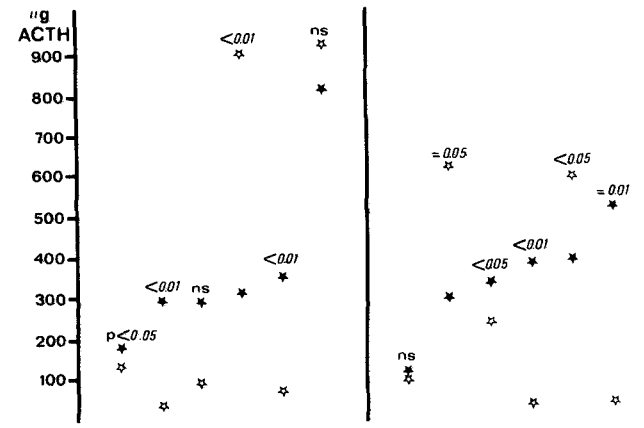

FIG. 3. Responses of 12 rats to a change of the initial concentration of ACTH solutions (★). (☆): On the left, responses to an increased concentration. On the right, responses to dilution.

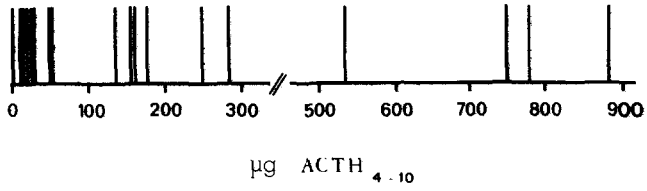

FIG. 4. Distribution of mean daily amounts of self-injected $ACTH_{4-10}$ in 18 rats.

parable to that obtained in the preceding experiment with $ACTH_{1-24}$. However, the individual mean daily amounts of intake were distributed over a broader range than that observed with the adrenally active analogue (Fig. 4).

DISCUSSION

The results of these three experiments demonstrate that rats offered to press a lever ad lib in their home cage, learn the operant response when reinforced by the intravenous delivery of either $ACTH_{1-24}$ or $ACTH_{4-10}$ analogue. The finding that 46% of rats, in the three experiments, did not learn the response is not surprising. In other self-administration experiments using reinforcers such as Insulin [15], a proportion of rats also failed to press the lever and thus did not exhibit a sustained self-injection pattern. The fact that in Experiment 1 only the rats which failed to press for ACTH also failed to press for NaCl solution suggests two possible interpretations. Either these rats were deficient in their learning abilities or, more likely, the implanted cardiac catheter produced a contingent painful stimulation, which could have counteracted the learning of a positive response.

When elicited, the lever-pressing for ACTH appears to be a specific response to the ACTH solution. This specificity is substantiated by three facts: (1) The daily amounts of ACTH solution obtained by rats were distributed over a relatively narrow range of doses. Such consistency contrasts with the wide interindividual variations observed in the same rats and commonly in others, with a control NaCl solution. (2) Rats of the first experiment when shifted from ACTH to saline or from saline to ACTH changed or maintained the response which confirmed the specificity of action for a particular dose range of ACTH. (3) The change in response in the majority of rats when the concentration of the solution was increased or decreased also confirmed that rats respond to the ACTH content of the solution and not to the vehicle. The self-administration of the adrenally inactive 4–10 analogue (Experiment 3) demonstrated that the self-administration of $ACTH_{1-24}$ in the two preceding experiments was not due to adrenocortical stimulation. Thus, it may be assumed that the reinforcing property of both analogues is related to the direct brain activity of the neuropeptide.

The self-injected doses of both analogues in each successive short bout are considerably higher than the amount of pituitary ACTH found and measured in the blood (100 pg/ml) [18]. However, the daily values of self-administration indicate that within each bout of lever-pressing and from bout to bout there exists an optimal positively reinforcing dose which results in a daily self-administration of 250 to 375 μg of $ACTH_{1-24}$ and 158 to 737 μg of $ACTH_{4-10}$. The lower and upper limits suggest a threshold and a ceiling of the positive reinforcing effectiveness. The upper limit also suggests a possible negative reinforcing or aversive effect of yet higher doses. An inverted U-shape curve of a dose-dependent facilitatory-inhibitory effect of post-trial injections of ACTH in either approach or avoidance learning, has been demonstrated by other investigators [12,14].

The finding that ACTH acts as a positive or eventually aversive reinforcer in a self-administration paradigm supports the hypothesis that the same reinforcing activity of ACTH is involved in the previously reported behavioral effects. This also indicates that the stress-induced release of ACTH can act directly on the brain not only by enhancing a motivational state but also by acting on brain rewarding or aversive systems. It has also been shown that rats previously treated with dexamethasone, which blocks pituitary ACTH release, demonstrated an impairment in their ability to learn an illness-induced taste aversion [13,19].

Whether ACTH acts on a diffuse brain rewarding system as observed during electrical self-stimulation and/or on specific receptor sites such as endogenous opiate receptors has yet to be determined. It has been suggested that ACTH interferes with opiate receptors and thereby modulates the aversive-pain mechanism. Preliminary results which indicate that Naloxone acutely and strongly inhibits ACTH intravenous self-administration are consistent with this interpretation.

REFERENCES

1. Bohus, B., W. H. Gispen and D. De Wied. Effect of lysine vasopressin and $ACTH_{4-10}$ on conditioned avoidance behavior of hypophysectomized rats. *Neuroendocrinology* **11**: 137–143, 1973.
2. Bohus, B. Pituitary adrenal influences on avoidance and approach behavior of the rat. *Brain Res.* **39**: 407–419, 1973.
3. Bohus, B., H. H. L. Hendrickx, A. A. Van Kolfschoten and T. G. Krediet. Effect of $ACTH_{4-10}$ on copulatory and sexually motivated approach behavior in the male rat. In: *Sexual Behavior: Pharmacology and Biochemistry*, edited by M. Sandler and G. L. Gessa. New York: Raven Press, 1975, pp. 269–275.

4. De Wied, D. and W. H. Gispen. Behavioral effects of peptides. In: *Peptides in Neurobiology*, edited by H. Gainer and J. L. Barker. New York: Plenum Press, 1977, pp. 391–442.
5. De Wied, D., A. Witter and H. M. Greven. Behaviourally active ACTH analogues. *Biochem. Pharmac.* **24:** 1463–1468, 1975.
6. De Wied, D. Effects of peptide hormones on behavior. In: *Frontiers in Neuroendocrinology*, edited by W. F. Ganong and L. Martini. London: Oxford University Press, 1969, pp. 97–140.
7. De Wied, D., A. Witter and S. Lande. Anterior pituitary peptides and avoidance acquisition of hypophysectomized rats. *Prog. Brain Res.* **32:** 213–220, 1970.
8. Dunn, A. J. and W. H. Gispen. How ACTH acts on the brain. *Biobehav. Rev.* **1:** 15–23, 1977.
9. Endröczi, E. Brain mechanisms involved in ACTH-induced changes of exploratory activity and conditioned avoidance behavior. In: *Neuropeptide Influences on the Brain and Behavior*, edited by L. H. Miller, C. A. Sandman and A. J. Kastin. New York: Raven Press, 1977, pp. 179–188.
10. Gispen, W. H., M. E. A. Reith, P. Schotman, V. M. Wiegant, H. Zwiers and D. De Wied. CNS and ACTH-like peptides: neurochemical response and interaction with opiates. In: *Neuropeptide Influences on the Brain and Behavior*, edited by L. H. Miller, C. A. Sandman and A. J. Kastin. New York: Raven Press, 1977, pp. 61–80.
11. Gispen, W. H. and V. M. Wiegant. Opiate antagonists suppress $ACTH_{1-24}$ induced excessive grooming in the rat. *Neurosci. Lett.* **2:** 159–164, 1976.
12. Gold, P. E. and R. Van Buskirk. Enhancement and impairment of memory processes with posttrial injections of adrenocorticotrophic hormone. *Behav. Biol.* **16:** 387–400, 1976.
13. Hennessy, J. W., W. P. Smotherman and S. Levine. Conditioned taste aversion and the pituitary-adrenal system. *Behav. Biol.* **16:** 401–412, 1976.
14. Isaacson, R. L., A. J. Dunn, H. D. Rees and B. Waldock. $ACTH_{4-10}$ and improved use of information in rats. *Physiol. Psychol.* **4:** 159–162, 1976.
15. Jouhaneau, J. and J. Le Magnen. Food related intravenous insulin self-administration in normal and diabetic rats. *Physiol. Behav.* **20:** 739–748, 1978.
16. Mirsky, I. A., R. E. Miller and M. Stein. Relation of adrenocortical activity and adaptative behavior. *Psychosom. Med.* **15:** 574–584, 1953.
17. Murphy, J. V. and R. Miller. The effect of adrenocorticotrophic hormone (ACTH) on avoidance conditioning in the rat. *J. comp. physiol. Psychol.* **48:** 47–49, 1955.
18. Ruhmann-Wennhold, A. and D. H. Nelson. Plasma ACTH levels in nonstressed and nonstressed adrenalectomized rats. In: *ACTH and Related Peptides: Structure, Regulation, and Action*, edited by D. T. Krieger and W. F. Ganong. New York: Academy of Sciences, 1977, pp. 498–508.
19. Smotherman, W. P. and S. Levine. ACTH and $ACTH_{4-10}$ modification of neophobia and taste-aversion responses in the rat. *J. comp. physiol. Psychol.* **92:** 22–23, 1978.
20. Steffens, A. B. A method for frequent sampling of blood and continuous infusion of fluids in the rat without disturbing the animal. *Physiol. Behav.* **4:** 833–836, 1969.
21. Van Riezen, H., H. Righter and D. De Wied. Possible significance of ACTH fragments for human mental performance. *Behav. Biol.* **20:** 311–324, 1977.
22. Wiegant, V. M., W. H. Gispen, L. Terenius and D. De Wied. ACTH-like peptides and morphine: interaction at the level of CNS. *Psycho-neuroendocrinology* **2:** 63–70, 1977.