Effects of Dopamine and ACTH on Steroid Sensitive Single Neurones in the Basal Hypothalamus

Previous reports from this laboratory have established the presence of dexamethasone sensitive single neurones in rat hypothalamus and midbrain[1,2]. Most of these neurones were inhibited by microelectrophoretically delivered dexamethasone-phosphate; a few were activated. These steroid sensitive neurones were also responsive to acetylcholine and L-noradrenaline. Acetylcholine predominantly activated these cells, whereas with L-noradrenaline inhibition rather than activation was observed. Furthermore, in preliminary studies carried out with ACTH, excitatory effects upon these cells were encountered. Recent investigations[3,4] have emphasized the role played by dopamine (3,4-dihydroxy-phenylethylamine) in hypothalamo-pituitary regulation. It was therefore decided to assess the responsiveness of steroid sensitive hypothalamic neurones to dopamine.

Dopamine (0.5–1 M), dexamethasone-phosphate (0.5 M) and synthetic ACTH (Tetracosactid) ($7 \times 10^{-4} M$) were delivered by microelectrophoresis[5,6] from 5-barrelled micropipettes to 11 Füllinsdorf albino rats (230–280 g body weight), anaesthetized by i.p. chloralose (75 mg/kg) and urethane (500 mg/kg). The amount of drug delivered is proportional to the intensity of current applied; 20–40 nA (nanoamperes) have been used in this experimental series. The spontaneous rate of discharge of single neurones was taken as the criterion for drug effects. Single neurones were marked by electrophoretic ejection of fast green[7] and identified histologically using the atlases of ALBEFESSARD et al.[8] and DE GROOT[9]. All neurones investigated in this series were situated in the medial basal (DMH, VMH, ARH) and anterior basal (AHA) hypothalamus.

Out of 49 neurones tested with dexamethasone-21-phosphate 9 were inhibited, 3 were activated and 37 remained unchanged. The time course of neuronal inhibition by dexamethasone-phosphate was no more uniform than it was in the former study[2]. Inhibition became apparent from 2–20 sec or more after the beginning of microelectrophoresis. The inhibitory effect of dexamethasone-phosphate (Figure 1A) exceeds the end of microelectrophoresis for variable times ranging from 5–100 sec.

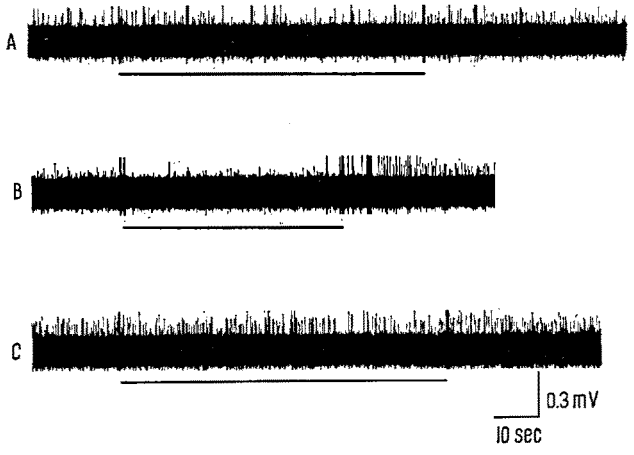

Fig. 1. Neuronal activity in the anterior hypothalamic area. (A) Partial inhibition by dexamethasone-phosphate (30 nA). (B) Strong inhibition by dopamine (30 nA). (C) Biphasic effects of ACTH (30 nA): activation followed by inhibition. — duration of microelectrophoresis.

Dopamine was tested on 9 dexamethasone-phosphate sensitive neurones. 7 out of these were strongly inhibited by dopamine, 2 remained unchanged. No activation was seen with this substance. The time course of inhibition with dopamine was very uniform. The neurones were inhibited by dopamine almost instantaneously after the start of microelectrophoresis, and the recovery time, 1–5 sec, was also short. In the example shown in Figure 1B, a sort of rebound effect is observed at the end of dopamine microelectrophoresis. Clearly the inhibitory effect of dopamine differs from that of dexamethasone-phosphate with respect to the time course. Furthermore, it is more intense than that of noradrenaline in the same region[2].

ACTH applied microelectrophoretically activates the steroid sensitive neurones (4 out of 5 cells). The time

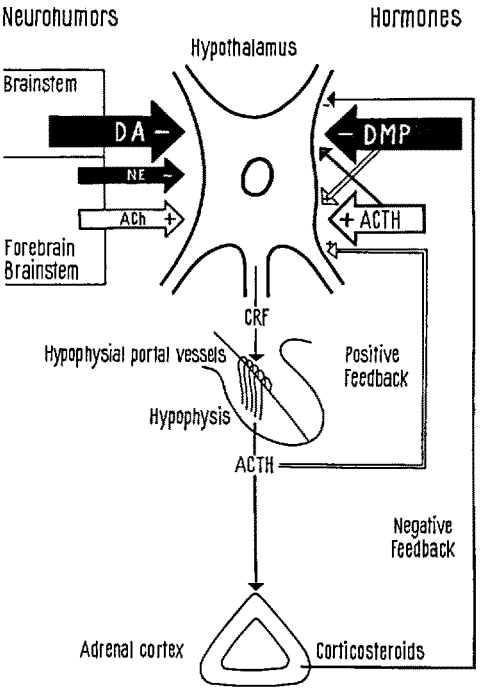

Fig. 2. Schematic interpretation of local effects of neurohumors and hormones on hypothalamic neurones, which may be involved in central control of ACTH secretion. This scheme may be valid under the condition that the CRF (corticotropin releasing factor) producing cells are sensitive to both neurohumors and hormones. DMP, dexamethasone-phosphate; DA, dopamine; NE, L-noradrenaline; ACh, acetylcholine. (NE and ACh effects have been reported previously.)

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course of ACTH activation is more or less uniform and shows a latency of 1–10 sec after the start of microelectrophoretic application; the recovery time is in the same order. Figure 1C demonstrates a biphasic effect of ACTH microelectrophoresis on neuronal activity. Initially a clear activation is recorded, which is followed by an inhibition of neuronal activity. When dopamine and ACTH were administered simultaneously to neurones activated by ACTH alone, the strong inhibitory effect of dopamine prevailed over the excitatory effect of ACTH (3 cells). Dexamethasone-phosphate insensitive neurones were never influenced by ACTH. Dopamine on the other hand inhibited the discharge rate of 7 out of 16 steroid insensitive neurones tested in the hypothalamus.

With the fast green method the position of the steroid sensitive and insensitive neurones was microscopically identified. All steroid sensitive neurones investigated in this series were situated in the medial basal hypothalamus, such as DMH (nucleus dorsomedialis hypothalami), VMH (nucleus ventromedialis hypothalami) and ARH (nucleus arcuatus hypothalami) and in the anterior hypothalamus (AHA — anterior hypothalamic area).

These experiments may demonstrate the dual sensitivity of certain hypothalamic nerve cells (Figure 2): (1) Neurohumoral sensitivity (dopamine): Experimental stimulation with dopamine might simulate the effect of dopamine containing nerve terminals. Dopamine could by inhibition regulate the trigger mechanism for the neurones producing the corticotropin releasing factor (CRF). (2) Hormonal sensitivity (ACTH, corticosteroid): Stimulation with these substances might simulate hormonal effects normally mediated by the blood. This mode of action of ACTH could represent a positive feedback mechanism[10] while the corticosteroid (dexamethasone-phosphate[11]) effect may represent a negative feedback mechanism in the regulation of ACTH production.

Zusammenfassung. Mit Hilfe der Mikroelektrophorese wurde die Wirkung von Dexamethasonphosphat, Dopamin und ACTH lokal an Einzelneuronen des Hypothalamus der Ratte geprüft. Dexamethason-phosphat (ein synthetisches Corticosteroid) hemmte mehrheitlich die Aktivität der steroidempfindlichen Zellen im Hypothalamus, einige wenige dieser Zellen wurden aktiviert. Dopamin hemmte die Aktivität dieser steroidempfindlichen Neuronen sehr stark. ACTH dagegen aktivierte diese Zellen. Diese Resultate werden in Zusammenhang mit einem negativen und einem positiven «Feedback»-Mechanismus, der die ACTH-Bildung steuert, diskutiert.

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The Effect of Prolonged Sympathetic Stimulation on Conduit Vessel Diameter

The disability of smooth muscle of resistance vascular bed to maintain sustained contraction at sympathetic stimulation, has been repeatedly reported[1–3]. Although the interpretations of the mechanisms underlying this phenomenon may vary, there seems to be little controversy in admitting the more or less pronounced role of accumulated tissue metabolites as a consequence of an impaired blood flow[4,5].

Thus it seems reasonable to presume that smooth muscle of vessels with no nutritional function, e.g. conduit vessels, should exhibit sustained contraction for any duration of stimulation and hence 'autoregulatory escape' should not be expected.

To investigate this problem, experiments were performed on 16 dogs, anaesthetized with thiopental sodium 60–70 mg/kg body weight. The peripheral end of the cut sympathetic trunk at the level of L_3–L_4 was stimulated with unipolar rectangular impulses of 5 msec duration, supramaximal amplitude and graded frequencies; each stimulation lasting 10 min. The diameter of the femoral artery (inductive transformer[6]) and pressure (electromanometer Elema) were recorded simultaneously.

As Figure 1A shows, at low frequency stimulation 1 imp/sec) the diameter of the femoral artery keeps contracting throughout the whole stimulation period. In contrast, at high frequency stimulation (15 imp/sec, Figure 1B) the diameter, after having reached minimum values (i.e. maximum response), tends to return to initial values in spite of continued stimulation.

The changes of diameter during stimulation at various frequencies, as related to the time axis of stimulation, were quantified and expressed as percentages of the maximum response of the respective series of stimulation. It is apparent in Figure 2 that at stimulation frequencies 1–2–4–8 imp/sec the diameter, having reached minimum values within 3–4 min of stimulation, varies insignificantly until the end of the stimulation. However, a gradual relaxation of the femoral artery occurred at 15 imp/sec stimulation, after contraction had reached its maximum within 4 min.

In view of existing discrepancies relative to release of the transmitter with prolonged sympathetic stimulation[7,8], it seemed less likely that the failure to maintain sustained contraction should be attributed solely to gradually decreasing amount of the transmitter at the site of its action. Thus, other mechanisms responsible for the revealed gradual decay of contraction had to be considered.

At first, the possibility was examined whether the gradual relaxation in spite of continued stimulation might

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