EFFECT OF PERITOL ON ACTIVITY OF THE HYPOTHALAMO–HYPOPHYSEO–ADRENAL SYSTEM

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Functional correlation has been demonstrated between the blood corticosteroid level and the intensity of serotonin metabolism [2, 10, 13]. The ability of serotonin to stimulate activity of the hypothalamo–adrenal system has been confirmed in experiments to determine the ACTH, corticosterone, and cortisol levels in the peripheral blood plasma of guinea pigs and rats [1, 4]. Elevation of the plasma ACTH and cortisol levels has been found also in man after administration of 150 mg of 5-hydroxytryptophan [6]. Elevation of the plasma corticosteroid level in man has been shown [14] to correlate with intensification of serotonin metabolism.

Peritol (cyproheptadiene), an antagonist of serotonin receptors, has begun to be used in clinical practice for the treatment of Cushing's disease [11, 12].

The object of the present investigation was to study activity of the hypothalamo–hypophyseo–adrenocortical system in rats under the influence of peritol and its dependence on the duration of administration and the dose of peritol.

EXPERIMENTAL METHOD

Male Wistar rats weighing 180-220 g were used. Peritol (cyproheptadine) (from Egut, Hungary) was given internally through a tube twice a day in a dose of 2 mg/100 g or 5 mg/100 g body weight in 0.5 ml distilled water. Blood levels of ACTH and corticosterone were determined in the animals 3 and 6 h and 14, 20, and 30 days after administration of peritol, using radioisotope kits from the Radiochemical Centre, Amersham (England), and aldosterone was assayed with the radioimmunologic kits from CEA-IRE-Sorin (France). Distilled water was given internally to the control animals in the same volumes.

EXPERIMENTAL RESULTS

After a single dose of peritol a marked decrease in the blood ACTH level began soon after administration (3 and 6 h; Table 1). However, the corticosterone and aldosterone concentrations in the animals increased compared with the control, despite the sharp fall in the ACTH level (Table 1). The absence of any decrease in the blood steroid hormone levels in rats receiving peritol, despite the marked fall in ACTH, was perhaps due to the considerable fall in the ACTH level during the first 3 h; according to some workers this may lead to a paradoxical response of the adrenals [9, 15]. The results of the present experiments confirmed the following hypothesis to some degree: 6 h after administration of peritol, during a further fall in the blood ACTH level of the animals, a decrease (compared with the previous period of the investigation) in the corticosterone and aldosterone concentrations also was observed (Table 1). However, the absence of any decrease in the blood corticosteroid levels in rats after a single dose of peritol may be attributable to the low concentration of the drug in the adrenal tissue. This was due to the much smaller number of serotonin receptors than in the brain, and, as a result, the minimally strong manifestation of the direct action of period on steroid production. Data have recently been published indicating that the action of peritol is linked with its effect not only on the activity of specific structures in the CNS and, correspondingly, on the pituitary trophic hormones and the endocrine glands which they control, but also directly on various tissues at the cellular level. The direct inhibitory action of cyproheptadine (peritol) on steroid production in the adrenals of patients with Cushing's disease has been described [8]. Similar data have been obtained on the direct inhibitory action of cyproheptadine on insulin and glucagon secretion [5, 7].

Further investigations also confirmed the writers' hypothesis that absence of a decrease in the corticosteroid concentrations after administration of peritol may perhaps be due to the low concentration of peritol in the adrenal tissue. This could be the result both of an inadequate dose of the drug and of its rapid elimination from the body. For instance, during prolonged administration of peritol to animals (for 14, 20, or 30 days) no changes were found in their blood corticosteroid

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TABLE 1. Blood ACTH, Corticosterone, and Aldosterone Levels in Rats Receiving Various Doses of Peritol (M ± m)

Time after administration of peritol, h	Number of animals	Dose of peritol, mg/100 g	Hormone concentration		
			ACTH, pg/ml	corticosterone, μg/100 ml	aldosterone, ng/100 ml
control (intact animals)	10	—	164±20	12,3±1,5	25,5±1,4
3	10	2	106±15 $P<0,05$	21,9±2,9 $P<0,001$	58,6±5,1 $P<0,001$
3	7	5	100±12 $P<0,02$ $P_1>0,5$	8,0±1,3 $P<0,05$ $P_1<0,001$	19,0±2,3 $P<0,05$ $P_1<0,001$
6	10	2	69±9 $P<0,001$	17,9±2,0 $P<0,05$	35,7±4,3 $P<0,05$
6	7	5	67±6 $P<0,001$ $P_1>0,5$	10,9±2,1 $P>0,5$ $P_1<0,05$	23,0±1,9 $P>0,5$ $P_1<0,05$

Legend. P) Significance of differences compared with control; P_1) significance of differences compared with animals receiving peritol in a dose of 2 mg/100 g.

TABLE 2. Blood ACTH, Corticosterone, and Aldosterone Levels in Rats after Receiving Peritol in a Dose of 2 mg/100 g Every 3 h (M ± m)

Time after administration of peritol, h	Number of animals	Hormone concentration		
		ACTH, pg/ml	corticosterone, μg/100 ml	aldosterone, ng/100 ml
control (intact animals)	10	164±20	12,3±1,5	25,5±1,4
6 h after administration of peritol in a single dose of 2 mg/100 g	10	69±9 $P<0,001$	17,9±2,0 $P<0,05$	35,7±4,3 $P<0,05$
6 h after administration of peritol in a dose of 2 mg/100 g every 3 h	7	40±2,7 $P<0,001$ $P_1<0,05$	7,5±1,4 $P<0,05$ $P_1<0,001$	17,0±2,3 $P<0,001$ $P_1<0,01$

Legend. P) Significance of differences compared with control; P_1) significance of differences compared with animals receiving a single dose of peritol.

and ACTH levels. The results now obtained also indicate that inhibition of activity of the hypothalamo–hypophyseo–adrenocortical system after administration of peritol is not connected with the duration of its administration but it is more likely to be due either to the use of higher doses of peritol or to the increased frequency of its administration, bearing in mind that the drug is rapidly excreted [3].

The next series of experiments were undertaken to verify these suggestions: Animals of one group received a single dose of 5 mg/100 g peritol internally, and their blood levels of corticosteroids and ACTH were determined 3 and 6 h later.

The animals of the second group received peritol in a dose of 2 mg/100 g every 3 h, and their blood corticosteroid and ACTH levels were determined 6 h after administration of the first dose. As Table 1 shows, when peritol was given in a dose of 5 mg/100 g the blood ACTH and corticosteroid levels of the animals were observed to be lower after 3 h than the corresponding values in the control group and also in the group of rats receiving peritol in a dose of 2 mg/100 g. However, 6 h after administration of peritol (5 mg/100 g), although the blood ACTH level of the rats was significantly lower than in intact animals, no decrease in the blood corticosterone and aldosterone levels of the experimental rats could be observed. Meanwhile, in the rats of this group the rise in the blood corticosteroid level found in animals receiving peritol in a dose of 2 mg/100 g was not observed (Table 2). If peritol was given in a smaller dose (2 mg/100 g), but more often (twice in the course of 6 h), a considerable and significant fall in the blood ACTH and corticosteroid levels of the animals was observed compared with their values in intact animals and also in rats receiving peritol in the same dose, but given only once (Table 2).

To obtain a marked inhibitory effect of peritol on functional activity of the hypothalamo-hypophyseo-adrenocortical system, it was thus necessary to give the drugs more often (every 3 h) in order to maintain its optimal concentration in the various tissues of the body, an essential factor in the realization of its effect both through central mechanisms (the ACTH concentration) and also as a result of the direct action of peritol on steroid production.

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NALOXONE-DEPENDENT WEAKENING OF EXCITATORY RESPONSES OF SNAIL NEURONS TO DOPAMINE BY MORPHINE

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The possible neurotransmitter and neuromodulator role of endogenous opiates is being widely discussed in the literature in connection with the use of various model systems [6, 13]. A promising way of studying this problem has been found to be by using molluscan neurons, on which opiate receptors have recently been identified by biochemical and electrophysiological methods [10-12]. The present writers have used neurons of the snail *Helix pomatia* to study the modulator functions of opiates in relation to spontaneous activity of the neurons [5] and their responses to artificially applied serotonin [1].

The aim of the present investigation, a continuation of previous studies, was to examine the effect of morphine on responses of snail neurons to dopamine.

EXPERIMENTAL METHOD

Experiments were carried out on neurons of *H. pomatia* from May through November. Neurons of all ganglia on the dorsal surface with stable discharges of high amplitude were used. The neurons were identified in accordance with Sakharov's classification [4]. For the microelectrode studies the apparatus from Nihon Kohden (Japan) was used. Potentials were recorded on a Recticorder RIG-4024 automatic ink writer. The recording microelectrode was filled with 2M potassium citrate. The cell membrane was polarized through the recording microelectrode by means of a bridge circuit. Dopamine (from Koch-Light, England), in a dose of $1 \cdot 10^{-7}$-$1 \cdot 10^{5}$ M, morphine hydrochloride in a dose of $1 \cdot 10^{-5}$ M, and naloxone (from Endo), in a dose of $1 \cdot 10^{-5}$ M, were injected into the perfusion fluid by means of a microsyringe. Otherwise the technique was the same as that described previously [1].

EXPERIMENTAL RESULTS

Of the 250 neurons studied (in 50 preparations) 120 cells responded to application of $1 \cdot 10^{-7}$-$1 \cdot 10^{-5}$ M dopamine by a marked reversible dose-dependent change in membrane potential. However, only those neurons (n = 35) whose responses to dopamine remained unchanged during frequent repeated applications were used in the experiments with morphine.

Of the 35 neurons (22 preparations), 19 cells responded to dopamine by depolarization of the membrane and quickening of the discharge, 14 responded by hyperpolarization and inhibition of the discharge, and in two cells the response was

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