EFFECT OF ETHYL p-CHLOROPHENOXYISOBUTYRATE ON THE ADRENOCORTICAL GLUCOCORTICOID FUNCTION IN GUINEA PIGS

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After daily administration of ethyl p-chlorophenoxyisobutyrate in a dose of 0.2 g/kg by mouth daily for 15-26 days to guinea pigs the concentration of 17-hydroxycorticosteroids (17-HCS) in the peripheral blood plasma fell. A fall in the excretion of individual 17-HCS in the urine took place chiefly on account of free unchanged cortisol, tetrahydrocortisol, and tetrahydro-11-deoxycortisol. An abundance of sudanophilic material was detected in the zona fasciculata of the gland. The test with ACTH showed that the existing functional reserves of the adrenal cortex were unchanged by the compound. The blood serum level of nonesterified fatty acids fell but the cholesterol level was unchanged. The possible mechanisms of the action of the compounds and prospects for its use in the treatment of hypercorticism are discussed.
KEY WORDS: ethyl p-chlorophenoxyisobutyrate; adrenal cortex..

Ethyl p-chlorophenoxyisobutyrate (clofibrate, miscleron, atromid S) has found a wide clinical application as one of the most active hypolipidemic and antisclerotic agents [5, 12, 14]. According to the available information [15], clofibrate in vitro inhibits the stimulating action of ACTH on steroid formation in the adrenals.

Considering that different types of hyperlipidemia require long treatment, it is important to evaluate the effect of this particular therapeutic substance on adrenocortical function in experiments in vitro, and the investigation described below was carried out for this purpose.

EXPERIMENTAL METHOD

Experiments were carried out on 18 male guinea pigs weighing 750-900 g. The animals were given ethyl p-chlorophenoxyisobutyrate* by mouth daily for 15-26 days in a dose of 0.2 g/kg, which gives a definite hypocholesteremic effect in rabbits with experimental hypercholesteremia [1]. The adrenocortical glucocorticoid activity was estimated from the peripheral blood plasma concentration of 17-HCS before and 2 h after intramuscular injection of 4 units normal-action ACTH [7]. At the same time, individual 17-HCS in the urine were determined chromatographically [2]. The serum levels of nonesterified fatty acids (NEFA) [9] and cholesterol [8] of the experimental animals were determined. At the end of the experiment the animals were killed and the adrenals were weighed and stained with hematoxylin–eosin and Sudan III and IV.

EXPERIMENTAL RESULTS AND DISCUSSION

The results in Tables 1 and 2 show that clofibrate lowered the plasma 17-HCS level by almost 2.4 times. The basal excretion of the total 17-HCS fractions in the urine was significantly reduced, mainly on account of free unchanged cortisol (F), the quantity of which fell by more than 60% of its original level. The concentrations of tetrahydrocortisol (THF) and tetrahydro-11-deoxycortisol (THS) also fell. The same trend also was

*The compound was synthesized in the authors' Institute by I. B. Simon and conventionally named "roxilate." However, in this translation the more usual term clofibrate will be used.

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TABLE 1. Plasma 17-HCS Concentrations (in μg %) before and after a Single Injection of ACTH during Clofibrate Administration (M ± m)

Time of investigation	Before ACTH injection	After ACTH injection	Increase	
			m mg%	in %
Before administration of clofibrate (initial values)	56,8±3,9	194,2±8,4	137,3±6,6	255,7±22,9
After administration of clofibrate	23,9±3,9	155,3±20,1	131,4±20,4	575,5±111,4
P	<0,001	<0,1	>0,7	<0,001

TABLE 2. Excretion of Cortisol and Its Metabolites (in μg/day) before and after Injection of Clofibrate (M ± m)

Time of investigation	THF	THE	F	THS	E	Total fractions without THS
Before administration of clofibrate (initial values)	15,7±2,0	9,9±1,6	110,8±16,4	4,4±1,3	11,0±3,6	147,5±19,5
After administration of clofibrate	7,6±1,4	6,1±1,4	47,1±7,6	1,3±0,5	5,9±1,96	65,9±8,4
P	<0,02	>0,1	<0,02	<0, 05	>0,1	<0,02

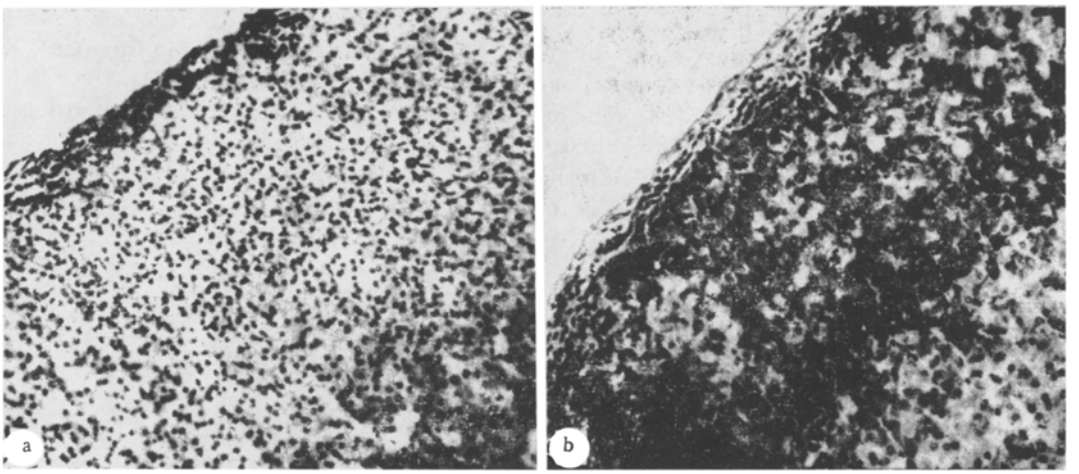

Fig. 1. Adrenal cortex of intact (A) guinea pig and guinea pig receiving clofibrate (B). Sudan IV, 100×.

established for the excretion of active cortisone (E) and its tetrahydro form (THE). The THF/THE and F/E ratios were unchanged.

The serum cholesterol concentration of the experimental animals was unchanged (86 ± 6.07 compared with 95 ± 16.09 mg % in the intact animals; $P > 0.5$) but the NEFA concentration was significantly reduced (from 0.64 ± 0.02 to 0.41 ± 0.01 meq/ml; $P < 0.001$). Since glucocorticoids play an important role in the mobilization of fatty acids [11, 13], the changes discovered in the NEFA concentration may be further evidence of the effect of the compound on adrenocortical function.

The response of the adrenals to a single injection of ACTH after administration of clofibrate was indistinguishable from that observed before. The increase in the response when expressed as a percentage can, however, be explained by the significantly lower plasma 17-HCS level before injection of the hormone [4]. Consequently clofibrate, although weakening glucocorticoid activity, does not change the existing reserves of the gland, a property that distinguishes it favorably from other inhibitors which depress the adaptive powers of the adrenals [3].

The microscopic structure of the adrenals of the experimental and control guinea pigs was identical (Fig. 1). Histochemical analysis of the sections revealed hypertrophy of the zona fasciculata of the gland tissue

as a result of distension of the cell cytoplasm with sudanophilic material. There were so many fat vacuoles that in places they joined to form yellow (Sudan III) and black (Sudan IV) drops. No changes in the content of sudanophilic material were found in either the zona reticularis or the zona glomerulosa. The absolute weigh to the adrenals of the animals receiving clofibrate was indistinguishable from the control.

Cholesterol is known to be synthesized in the adrenals in guinea pigs [3] and not to be taken up from the plasma. Depression of adrenocortical function discovered in the animals of this species, at a time of unchanged serum cholesterol concentration, is thus evidence that clofibrate does not exert its influence on 17-HCS biosynthesis at the stage of cholesterol formation. The less frequent discovery of the 11-deoxycortisol fraction (one of the precursors of F in the adrenal cortex of guinea pigs [6]) and of excretion of its principal metabolite (THS) can be regarded as indirect evidence that the compound acts on the early stages of corticosteroid formation. Considering that adrenal hypertrophy also was absent in the experimental animals, the possibility of an indirect action of clofibrate through the pituitary likewise cannot be ruled out.

A definite inhibitory effect of clofibrate on the glucocorticoid function of the adrenal cortex was thus established during its administration for a comparatively short time to guinea pigs. To decide whether this therapeutic substance, which has virtually no general toxic action [10, 16], should be used for the treatment of hypercorticism, suitable clinical trials must be undertaken.

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