EFFECT OF ALLERGIC ALTERATION ON INTENSITY OF ENZYMIC INACTIVATION OF CORTISOL IN THE DOG KIDNEY

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UDC 616-056.3-07 : 616.61-008.944.53

Injection of reacting doses of specific allergen into the fluid perfusing the kidneys of sensitized dogs caused no change in the intensity of cortisol metabolism. Since the same doses of specific allergen severely disturbed the intensity of cortisol metabolism in the liver of the same dogs, it is postulated that not all the enzymes concerned with cortisol metabolism are affected by allergic alteration of organs, but only some of them, notably Δ^4-cortisol reductase, through a reversible change in the conformation of its apoenzyme.

The rate of disappearance of exogenous cortisol and corticosterone from the blood stream is reduced in some allergic diseases [3, 4], indicating depression of the metabolism of these hormones. In experiments on dogs Pytskii and Gulyi [1] showed that allergic alteration lowers the intensity of cortisol metabolism in the liver. However, the possible disturbances on metabolism of this hormone in other tissues has not yet been studied.

The possibility of a disturbance of cortisol metabolism in allergic reactions of nondelayed type was investigated in dog kidneys.

EXPERIMENTAL METHOD

The kidneys were perfused in vitro with Tyrode solution containing 1.2% dextran. Cortisol was added to the artificial perfusion fluid in concentrations varying from physiological to stressor. The fluid flowing from the kidney was collected in portions every 9 min, and the content of residual unmetabolized cortisol was determined in each portion by a fluorometric method. The fluorometer designed by Pytskii (incandescent lamp as the stabilized light source, primary and secondary interference filters 470 and 540 nm, FÉU-17a recorder with mirror galvanometer) was used for the determination. The rate of perfusion was maintained at about 10 ml/min/100 g weight of kidney. Collection of the perfusion fluid began after washing out for 15 min. In most experiments perfusion continued for 6-8 h. The intensity of metabolism in each case was judged as a rule from the residual cortisol concentration in the outflowing perfusion fluid, and also after analysis of the results as a whole and comparison of those for the individual dogs, from the quantity of metabolized cortisol in μg/min/100 g weight of kidney at equal rates of perfusion. The metabolites of cortisol were isolated by paper chromatography in a $BuSh_5$ system and identified from their UV fluorescence, their R_f values, their chromogen spectrum in sulfuric acid, and reactions with phenylhydrazine and blue tetrazolium. Sensitization with normal horse serum (NHS) was carried out with three injections at intervals of 24 h, in doses of 0.5 ml NHS/kg body weight. Sensitization with egg albumin was carried out by three subcutaneous injections of a mixture of egg albumin and complete adjuvant in doses of 1.5 ml egg albumin and 1 ml adjuvant/10 kg body weight.

Allergy Research Laboratory, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR, A. D. Ado.). Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 72, No. 12, pp. 25-27, December, 1971. Original article submitted January 5, 1971.

TABLE 1. Effect of Injections of Reacting Doses of Specific Allergens on Intensity of Cortisol Metabolism in Kidneys of Dogs Sensitized with NHS and CEA

Dog No.	Concn. of allergen/ml perfusion fluid		Cortisol concn. in inflowing perfusion fluid	Cortisol conc. in outflowing perfusion fluid (in μg%)		
	NHS (in ml)	CEA (in μg)		before injection	during injection	in first portion after injection
110	0,005	—	85,0	64,9	64,0	64,9
111	0,005	—	74,5	64,2	64,2	62,2
126	0,01	—	10,9	5,43	5,46	6,22
73	0,017	—	20,2	13,05	13,4	13,2
76	0,096	—	19,0	14,4	16,1	14,0
75	0,095	—	21,0	14,1	16,6	14,6
126	0,097	—	10,9	5,2	5,9	5,0
126	0,11	—	10,9	5,7	5,8	5,6
76	0,135	—	19,0	14,0	15,9	13,1
83	—	81,0	10,5	4,55	4,64	4,70
83	—	88,0	10,5	4,57	4,50	4,54
106	—	88,0	10,65	4,50	4,48	4,58
108	—	100,0	17,50	9,27	10,05	10,05
83	—	106,0	10,50	4,55	4,55	5,17
109	—	125,0	16,80	8,64	8,60	8,80
72	—	370,0	22,0	16,20	17,60	17,10
107	—	625,0	10,7	3,24	3,12	3,35
107	—	1011,0	10,7	3,05	3,20	3,05

EXPERIMENTAL RESULTS

Altogether 41 mongrel dogs were used. In the experiments of series I on seven intact dogs certain features of cortisol metabolism in the kidneys of the animals under the experimental conditions used were investigated.

The results showed that if the kidneys were perfused with solution not containing cortisol, no substances giving fluorescence which could interfere with the estimation of cortisol were present in the outflowing perfusion fluid after 15 min.

With a constant concentration of cortisol in the inflowing perfusion fluid the intensity of its metabolism was constant throughout the period of perfusion. With an increase in the cortisol concentration in the inflowing perfusion fluid, however, the rate of its metabolism was increased. For instance, if the cortisol concentration increased from 4.10 to 99 μg%, the intensity of its metabolism rose from 0.277 to 2.00 μg/min/100 g. It can be concluded that a passive self-regulating control mechanism operates in the kidneys, just as in the liver [2, 5].

The quantity of Porter–Silber chromogens was not increased after β-glucuronidase hydrolysis, i.e., the dog's kidneys did not form glucuronides of this group of corticosteroids. No tetrahydro-derivatives of cortisol were formed in the kidneys and its principal metabolite was cortisone. If the cortisole concentration in the inflowing perfusion fluid ranged from 17.4 to 80.5 μg%, the quantity of cortisone formed was 11.6-15.0%.

In the experiments of series II on the kidneys of nine intact dogs, the effect of NHS and crystalline egg albumin (CEA) on the intensity of cortisol metabolism was studied. The results showed that NHS in concentrations up to 400 μg/ml caused no change in the intensity of cortisol metabolism.

Injection of the specific allergen in concentrations up to 0.02 ml/ml perfusion fluid entering the kidneys of dogs sensitized with NHS caused no change in the intensity of cortisol metabolism, but in concentrations of between 0.10 and 0.13 ml/ml it lowered the intensity of metabolism very slightly in a few cases only. Moreover, this effect was seen only at the moment of injection of the NHS, after which the intensity of cortisol metabolism returned to its original level in the first portion collected after injection of the allergen (Table 1). It is interesting to note that these concentrations of NHS gave rise to a marked and prolonged decrease in the intensity of cortisol metabolism in the liver of the same dogs [1].

A similar reaction to injection of CEA was observed in the kidneys of dogs sensitized with egg albumin. CEA concentrations of up to 200 μg/ml, which caused a very marked decrease in the intensity of cortisol metabolism in the dog's liver, had no effect whatsoever on their kidneys. Large concentrations of CEA likewise had virtually no effect (Table 1).

In the experiments of Pytskii and Gulyi [1], in which reacting doses of allergens were injected into the liver of similarly sensitized dogs, corresponding doses of specific allergen caused a sharp decrease in the intensity of cortisol metabolism accompanied by a decrease in the formation of the principal cortisol metabolite–tetrahydrocortisol. This indicates inhibition of Δ^4-cortisol reductase activity. Injection of NADP·H_2, the cofactor of this enzyme, in these experiments restored Δ^4-cortisol reductase activity only negligibly. The results of the present experiments, showing that cortisol metabolism in the kidneys of the sensitized dogs, unlike in the liver, is undisturbed, coupled with the known fact that Δ^4-cortisol reductase is absent from the dog kidney, suggests that a mechanism for depressing the activity of Δ^4-cortisol reductase exists in the liver. The chief cofactor of the other enzyme system participating in cortisol metabolism is also known to be NADP·H_2, but in the kidneys no disturbance of cortisol metabolism was revealed. Consequently, the factor inhibiting the effect of the allergen–antibody reaction is not NADP·H_2 deficiency, but evidently a change in the conformation of the Δ^4-cortisol reductase apoenzyme.

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