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THE INFLUENCE OF KININS ON THE METABOLIC EFFECTS OF CHOSEN DRUGS*

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Abstract—The influence of bradykinin and kallikrein on some metabolic effects of catecholamines and theophylline were studied. The activity of phosphorylase *a* in heart muscle, the level of free fatty acids in the serum and epididymis, and the lactic acid content in the blood, striated muscle and intestine of the rat were studied after the administration of all drugs *in vivo*. Furthermore, the content of free fatty acids in the epididymal adipose tissue was estimated after incubation with the drugs *in vitro*. It was found that neither bradykinin nor kallikrein administered alone altered the studied parameters. However, kinins significantly diminished the effects of norepinephrine, epinephrine, isoprenaline and theophylline on the metabolic indicators. The authors suggest that kinins could act as modulators of the activity of adrenergic receptors and this modulating action could depend on their effect on cyclic 3′,5′AMP.

An increased activity of kinin-forming enzymes and elevation of kinin level was observed in a number of physiological and pathological states [1–3].

It was found that kinins release catecholamines from the adrenal glands and from tissue stores [4, 5] and that they play an essential role in the action of catecholamines [6].

It seemed to be of interest to investigate the influence of kinins on some characteristic metabolic effect of catecholamines, i.e. the activity of phosphorylase *a* in the heart muscle, the level of free fatty acids in the serum and epididymis, and the lactic acid content in the blood and tissues.

MATERIALS AND METHODS

Male Wistar rats weighing about 200 g were used in these studies. The animals were fed with a standard diet and given water ad lib.

Norepinephrine (norepinephrine bitartate, Sigma Chemical Co.) in a dose of 500 μg/kg, epinephrine (epinephrine bitartate, Sigma Chemical Co.) in a dose of 500 μg/kg and isoprenaline (isoprenaline sulphate, Boehringer) in a dose of 50 μg/kg were given intraperitoneally (i.p.). The effects of these drugs were determined 10 min after administration. Theophylline (theophyllinum, Polfa) in a dose of 100 mg/kg was given i.p. 30 min before decapitation. Bradykinin (bradykinin, Reanal, Budapest) in doses of 0.25 μg, 1.0 μg, 2.5 μg and 7.5 μg/kg was given intravenously (i.v.) simultaneously with the drugs. Kallikrein (kallikrein, Winthrop) in doses of 150 and 200 units/kg was injected 40 min before drug administration, because it was found that at that time the activation of kinin-forming enzymes by kallikrein is maximal [7]. The animals were anesthetised by i.p. injection of sodium hexobarbital in dose of 150 mg/kg, 15 min before being sacrificed.

The heart muscle was excised and phosphorylase *a* activity was measured by the method of Diamond and Brody [8] as modified by Robak and Gryglewski [9]. Inorganic phosphate was determined by the method of Fiske and Row [10]. The activity of phosphorylase was expressed in μmoles of inorganic phosphate released during the incubation per g of tissue per min.

The percentage of phosphorylase *a* activity was calculated from the enzyme activity in the samples without 5′AMP and in those with 5′AMP. Since none of the substances tested significantly influenced the level of total phosphorylase *a* the results were expressed in percentage of phosphorylase *a* activity. Glycogen was purified using the method of Sutherland and Wosilait [11].

Free fatty acids in the serum *in vivo* and in the epididymis fat tissue incubated *in vitro* were determined by the method of Novák [12]. The adipose tissue was isolated from the rat epididymis according to the Winegrad and Renold technique [13]. Each portion of tissue was incubated for 3 hr in 5 ml of 5% bovine albumin in 0.15 M phosphate buffer of Krebs–Ringer at pH 7.4 and 37°. The medium was equilibrated with 95% oxygen–5% carbon dioxide both before and after addition of the tissue. The following substances were added to the incubating medium: epinephrine, 10 μg; norepinephrine, 10 μg isoprenaline, 1 μg; theophylline, 3 mg; kallikrein, 20 U, bradykinin, 10 μg. The amount of released free fatty acids was calculated by subtraction of their content in the incubating medium before incubation from that found after incubation and was expressed in μeq/g of tissue/3 hr. The concentration of free fatty acids in the serum was expressed in μeq/l.

Lactic acid in the blood, striated muscle and intestine was determined by the method of Ström [14] and expressed in mg%. The results were statistically analysed by the Student *t*-test. The final results represent the average values of 6–15 readings including standard deviations (2 S.D.).

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Table 1. The effect of various doses of bradykinin on phosphorylase *a* activity in heart muscle of rat after norepinephrine adminstration

Ordinal number	Drugs	Number of experiments (n)	Phosphorylase *a* activity in heart muscle (% ± S.D.)	Statistical significance P
1	Control (saline)	12	22.0 ± 4.5	
2	Bradykinin 0.25 μg	8	19.5 ± 8.1	
3	Bradykinin 1 μg	8	19.0 ± 4.2	
4	Bradykinin 2.5 μg	6	21.0 ± 5.1	
5	Bradykinin 5 μg	6	22.0 ± 4.4	
6	Bradykinin 7.5 μg	12	20.0 ± 4.5	
7	Norepinephrine	8	54.0 ± 11.5	$P_{1-7} < 0.001$
8	Norepinephrine + bradykinin 0.25 μg	8	51.0 ± 8.0	
9	Norepinephrine + bradykinin 1 μg	8	48.0 ± 6.0	
10	Norepinephrine + bradykinin 2.5 μg	6	40.0 ± 4.2	$P_{7-10} < 0.01$
11	Norepinephrine + bradykinin 5 μg	6	34.0 ± 10.0	$P_{7-11} < 0.01$
12	Norepinephrine + bradykinin 7.5 μg	9	34.0 ± 9.1	$P_{7-12} < 0.001$

RESULTS

Bradykinin given in doses of 0.25–7.5 μg/kg did not have any effect on phosphorylase *a* activity in the heart muscle of the rat (Table 1), but norepinephrine increased the activity of this enzyme. Bradykinin administered together with norepinephrine decreased the stimulatory effect of norepinephrine on the activity of this enzyme. Beginning with a dose of 2.5 μg/kg, statistically significant effects of bradykinin were observed.

It was found that high doses of kallikrein (150–200 units/kg) did not change the activity of phosphorylase *a* (Table 2). On the other hand, bradykinin (7.5 μg/kg) and kallikrein (200 units/kg) lowered the stimulatory action of epinephrine, norepinephrine and isoprenaline on phosphorylase *a* activity.

Bradykinin and kallikrein did not affect the level of free fatty acids in the epididymis and in the serum of rat (Table 3). Increase in free fatty acid content was observed after administration of epinephrine or norepinephrine. Isoprenaline did not have such an effect. This action of epinephrine and norepinephrine was diminished by administration of bradykinin or kallikrein.

Bradykinin and kallikrein did not affect the level of lactic acid in the blood, skeletal muscle and intestine of the rat (Table 4). On the other hand, a diminishing effect of bradykinin and kallikrein on catecholamine-induced increase of lactic acid content in the investigated organs was observed.

As can be seen from Table 5, theophylline increases the phosphorylase *a* activity in the heart muscle and the level of free fatty acids in the serum and epididymis. These effects of theophylline were reduced by simultaneous administration of bradykinin.

Isoprenaline also increases the phosphorylase *a* activity and this effect is reduced by bradykinin administered alone or together with theophylline.

DISCUSSION

Our investigations showed that bradykinin administration or activation of the kinin-forming enzymes in animals under the influence of kallikrein did not change the activity of phosphorylase *a*, the level of fatty acids in the epididymis and serum or the level of lactic acid in the blood, striated muscle and intestine of the rat. We have confirmed the observations of other authors [9, 15] that the administration of adrenergic receptor stimulating drugs (epinephrine, norepinephrine, isoprenaline) results in the increase of phosphorylase *a* activity in heart muscle of rat, and causes the increase of the level of free fatty acids in serum and epididymis, and increases the level of lactic acid in tissues. The observed increase of the free fatty acids level after administration of isoprenaline was not statistically significant but it could result from lower sensitivity of adrenoreceptors in the rat fatty tissue on the action of isoprenaline [16]. Both kallikrein and bradykinin decrease the metabolic effects enhanced by norepinephrine, epinephrine and isoprenaline.

It is well known that the metabolic effects of catecholamines are mediated by cyclic 3′5′AMP, an important regulator of intracellular processes [17–20].