MECHANISM OF ACTION OF CYCLOKININS

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The writers showed previously [3, 4, 6] that, unlike natural linear kinins, such as bradykinin (BK) and kallidin (K), which have a brief hypotensive effect and marked myotropic activity, their cyclic analogs (CBK and CK, respectively) have a powerful and prolonged hypotensive action in experiments on anesthetized rats and weak myotropic action on the isolated rat uterus. It has been suggested that the more prolonged hypotensive effect of the cyclic kinins than of their linear analogs is due to a change in the rate of their metabolism and with the possible release of mediators by the cyclic kinins capable of inducing a vasodepressor effect or of disturbing the balance between systems controlling the arterial pressure (BP). This paper describes the results of a comparative study of the mechanism of the myotropi- and hypotensive effects of the following linear and cyclic kinins: Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg (BK), Lys-Pro-Pro-Gly-Phe-Gly-Pro-Phe-Arg-(CBK), and Lys-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg- (CK).

EXPERIMENTAL METHOD

In experiments *in vitro* isotonic contractions of the uterus were recorded by means of a semiautomatic six-channel electronic system [1, 2] in the presence of the most effective concentrations of BK (10^{-8} M) and CK (10^{-5} M), in the absence and in the presence of the following "analyzer" substances (preliminary incubation of the uterus for 3 min): hydrochlorides of phentolamine (10^{-5}-10^{-4} g/ml), propranolol (10^{-7}-10^{-6} g/ml), papaverine, atropine sulfate, dimedrol, and verapamil (10^{-7}-10^{-5} g/ml), and methysergide (10^{-6}-10^{-5} g/ml).

In experiments *in vivo* the effect of BK, CBK, and CK on BP was studied in anesthetized and unanesthetized rats with different initial BP levels: normotensive and genetically hypertensive rats, rats with renal hypertension, and also anesthetized guinea pigs and cats.

Albino rats of both sexes weighing 180-200 g were used. BP was recorded in the common carotid artery of rats anesthetized with urethane (0.5 ml of a 25% solution/100 g body weight, intraperitoneally), by means of a Bentley Trantec Physiological Pressure Transducer, on a two-channel "Gemini" recorder (Ugo Basile, Italy). The systolic pressure (SP) of unanesthetized rats was measured by plethysmography, using pneumatic pulse transducer and Physiograph (Narco Biosystems, USA); the ECG and respiration also were recorded. To determine the effect of CBK and BK on BP of genetically hypertensive rats, male Aoki-Okamoto rats [9] aged 12 months with SP of 200-225 mm Hg were used. To obtain a model of renal hypertension unilateral nephrectomy was performed on the right kidney of rats anesthetized with hexobarbital and the left kidney was wrapped in cellophane [10]. The rats were used in the experiments 2 weeks after the operation, when SP was 170-190 mm Hg.

BP of cats of both sexes weighing 3.3-3.8 kg, anesthetized with chloralose (90 ml/kg, intraperitoneally), was recorded electromanometrically from the common carotid artery on the Physiograph; the ECG and respiration also were recorded.

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TABLE 1. Effect of "Analyzer" Substances on Myotropic Effects of BK and CK

Concentration of substance, g/ml	Myotropic activity, %	
	BK (10^{-8} M)	CK (10^{-5} M)
Phentolamine	100	100
$3{,}3 \cdot 10^{-5}$	91,25±3,15	67,80±2,08
$6{,}6 \cdot 10^{-5}$	83,33±1,87	35,90±0,18
10^{-4}	52,70±0,68	0
Methysergide		
$3 \cdot 10^{-6}$	86,80±0,13	66,67±3,14
10^{-5}	74,32±0,57	35,59±0,25
Papaverine		
10^{-7}	75,96±0,23	78,57±1,62
10^{-6}	68,92±0,14	68,97±0,59
10^{-5}	14,64±7,88	0
Verapamil		
10^{-7}	31,67±0,24	0
10^{-6}	12,50±0,35	0
10^{-5}	0	0
Atropine		
10^{-7}	101,58±0,67	89,29±3,89
10^{-6}	97,93±0,95	100,00±1,15
10^{-5}	101,51±1,87	98,41±0,86
Propranolol		
$7 \cdot 10^{-7}$	99,33±0,67	110,48±6,34
$1{,}3 \cdot 10^{-6}$	100,93±1,05	98,65±4,15
Dimedrol		
10^{-7}	110,00±5,60	110,48±7,84
10^{-6}	100,00±2,70	105,71±2,13
10^{-5}	91,35±5,13	97,62±0,92

Male guinea pigs weighing 310-350 g were anesthetized with urethane (1.5 g/kg, intraperitoneally); BP, the ECG, and respiration were recorded on the Physiograph.

The substances for testing were injected into the femoral vein separately, in a volume of 0.1 ml/200 g body weight in doses of 0.5-500 μg/kg. In the experiments on anesthetized rats to study the mechanism of the hypotensive effect of BK and CK, "analyzer" substances (atropine sulfate, propranolol hydrochloride, indomethacin, dimedrol, and methysergide in a dose of 1 mg/kg and $CaCl_2$ in a dose of 100 mg/kg) were injected 1 min before administration of the kinins.

The effect of BK and CK on the blood flow was studied in experiments on the isolated rabbit ear connected to a perfusion apparatus. Ringer's solution for warm-blooded animals, of the following composition (in g/liter): NaCl 9.0, KCl 0.42, $CaCl_2$ 0.24, $NaHCO_3$ 0.5, glucose 1.0, was passed through a cannula inserted into a vein of the ear. CBK and BK, in a concentration of 10^{-7} M, were introduced through the cannula with the nutrient solution.

The effect of BK, CBK, and CK on kininase II activity was determined over a concentration range from 10^{-8} to 10^{-4} M [5] by a fluorometric method [8]. Whole human and rat blood serum, in which the initial kininase II activity, determined as the rate of removal of histidyl-leucine from the standard substrate, was 32.36 and 80.08 nmoles/ml/min, respectively, and was taken in each separate case as 100, was used.

For statistical analysis of the results each value was calculated as the mean for six experiments ± the standard error.

EXPERIMENTAL RESULTS

The data in Table 1 show that phentolamine and papaverine hydrochlorides, methysergide, and verapamil inhibited the myotropic effects of BK and CK in experiments on the isolated rat uterus. Atropine, dimedrol, and propranolol hydrochloride did not affect the myotropic effects of BK and CK. Despite the difference in affinity of BK and CK for smooth muscle receptors of the rat uterus (values of specific affinity pD_2 are 9.07 and 6.39, respectively [3]), their myotropic effects are evidently produced by a similar mechanism: partly directly, partly through α-adrenergic and serotoninergic systems, but mainly through Ca^{++}-dependent systems.

In experiments on anesthetized normotensive and genetically hypertensive rats and on rats with renal hypertension CBK in a dose of 50 μg/kg considerably lowered BP (by 45%) during more than 3 h, caused marked bradycardia (by 71%) for 1 min, but had no significant effect on the amplitude and frequency of respiration.

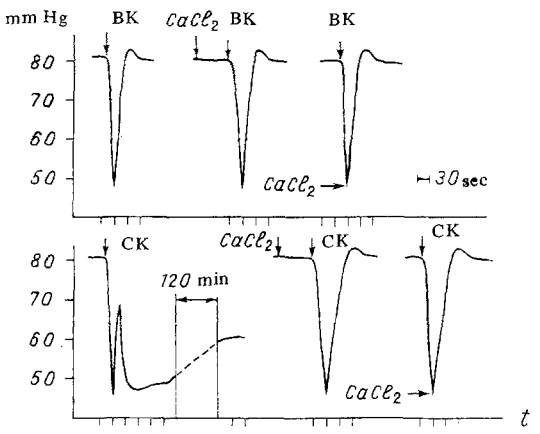

Fig. 1

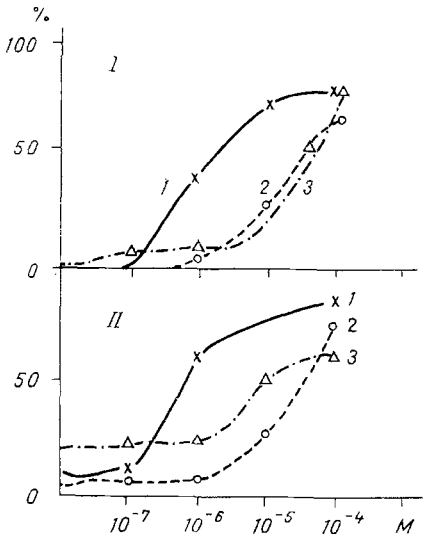

Fig. 2

Fig. 1. Scheme showing influence of $CaCl_2$ (100 mg/kg) on hypotensive effects of BK and CK (50 μg/kg) in experiments on anesthetized normotensive rats.

Fig. 2. Inhibition of action of kinase II on rat (I) and human (II) blood serum. BK (1), CBK (2), and CK (3).

Indomethacin, dimedrol, and methysergide did not affect the hypotensive effects of BK and CK in experiments on anesthetized normotensive rats. This indicates that the hypotensive effects of BK and CK are not realized through the release of mediators such as prostaglandins, histamine, and serotonin. $CaCl_2$ likewise had no significant effect on the hypotensive effect of BK and CK, but reduced the duration of the hypotensive effect of CK considerably (Fig. 1).

Although the exact role of Ca^{++} ions in realization of the hypotensive effect of CK is not clear, it is evident that Ca^{++}-dependent systems are somehow responsible for prolonging the hypotensive effect of CK. It may be that CK affects the mechanisms of Ca^{++} ion transport and, in particular, that it reduces the intracellular Ca^{++} concentration, which leads to prolonged relaxation of the vascular smooth muscle.

In experiments on unanesthetized normotensive rats CBK in a dose of 50 μg/kg lowered SP by 10-14% in the course of 24 h; the heart rate increased in the course of 1 h by 7% compared with initially. In experiments on unanesthetized genetically hypertensive rats CBK in a dose of 50 μg/kg lowered SP by 13% without any significant change in pulse rate for 1 h; BK in the same dose lowered SP by 9% in the course of 10 min. CBK (25 μg/kg) lowered SP sharply (by 34%) in the course of 1 h in unanesthetized rats with renal hypertension, and at the same time the pulse rate was increased by 16%.

In experiments on anesthetized cats and guinea pigs CBK in doses of up to 100 μg/kg had no significant effect on BP or the pulse rate, evidence that its action is species-specific.

In experiments on the isolated rabbit ear CBK and BK reduced the volume of fluid flowing through the vessels equally (by 22 and 25%, respectively), the effect being maximal after 5-7 min.

CBK and CK inhibited kininase II activity (ID_{50}) in human and rat blood serum about equally (inhibitory action $ID_{50} = 10^{-5}$ M; Fig. 2). ID_{50} of BK was higher than that of the cyclic kinins, namely 4.53×10^{-7} and 2×10^{-6} M for human and rat blood serum, respectively. The greater inhibitory activity of BK may perhaps be explained by the presence of a "free" Arg-Pro-Pro fragment, not incorporated into the ring, and which, according to data in the literature [7], is a relatively powerful inhibitor of kininase II.

The results thus indicate that the mechanisms of the myotropic and hypotensive action of linear and cyclic analogs of BK and K act in the same direction, but the main difference determining prolongation of the hypotensive action of CK is evidently participation of Ca^{++}-dependent systems in its hypotensive effect.

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EFFECTIVENESS OF LITHIUM HYDROXYBUTYRATE IN RESERPINE DEPRESSION

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In view of data in the literature on the therapeutic value of lithium salts in depression [3, 14, 15] and of the specific features of the psychotropic profile of lithium hydroxybutyrate [2, 4, 7, 8] it was decided to investigate the effectiveness of this compound on a model of reserpine depression.

EXPERIMENTAL METHOD

Experiments were carried out on 35 rabbits weighing 2.5-3.0 kg with electrodes implanted permanently by the technique described previously [8]. Electrodes were inserted into the frontal and occipital regions of the cortex, the basal nuclei of the amygdala, head of the caudate nucleus, dorsal hippocampus, and posterior hypothalamus. Brain electrical activity was recorded on an 8-channel Orion electroencephalograph and analyzed by the method of narrow-band analog filtration, using an analyzer and integrator from Estergom. The powers of the δ-, θ-, α-, β_1-, β_2-, and γ-rhythms, components of the whole electroencephalogram (EEG), were determined in a 10-sec integration interval. In each experiment no fewer than three EEG cuts were integrated for each brain structure, so that the total time of EEG analysis was 5 min or more. The initial data were rated at zero.

Rhythm binding to flashes of different frequencies, applied by an FS-02 photostimulator, served as the indicator of the functional state of the visual cortex.

Conditioned reflex experiments were carried out on rats weighing 180-200 g. A conditioned avoidance reaction (CAR) was formed in the animals in a special chamber with electric floor and a vertical rod. The conditioned stimulus was acoustic (a bell ringing for 5 sec). The time between conditioned and unconditioned (electric shocks through the floor, 50-80 V, 5 sec) stimuli was not more than 0.5 sec, and between individual tests it was 20 sec. The conditioned reflex was considered to have been formed if eight out of ten responses were positive.

Lithium hydroxybutyrate (10 mg/kg) or distilled water (1 ml/kg, control) was injected subcutaneously daily at the same time for 1 week. On the 8th day reserpine (in the form of rausedil) was injected subcutaneously into the animals in a dose of 0.125 mg/kg. An additional control series was set up to estimate the effects of reserpine: rabbits and rats were injected

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