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RAT UTERINE CONTRACTION BY KALLIKREIN AND ITS DEPENDENCE ON UTERINE KININOGEN

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Abstract—Smooth muscle responses to kallikrein (EC 3.4.21.8) are generally considered to result from kinin formation. In the present study, this premise was reexamined with respect to the isolated rat uterus. Rat submandibular gland kallikrein produced contractions of the rat uterus but the contractions disappeared after successive additions of the same dose of the enzyme to the preparation. Kallikrein-induced rat uterine contractions as well as bradykinin-induced contractions were enhanced by rat submandibular gland bradykinin potentiating factor. The incubation of kallikrein with rat uterine extract in the presence of a kininogen-depleted rat uterus produced kinin which elicited the uterine contraction. An extract from uterine horns previously depleted of kininogen was prepared. Incubation of this extract with kallikrein in a bath containing a kininogen-depleted rat uterus did not evoke uterine contraction. The incubation of four rat uterine horns with kallikrein in the presence of a uterine horn previously depleted of kininogen elicited contractions of the depleted uterus. These results suggest that the contraction produced by kallikrein involves kinin release from the uterus.

Rat glandular kallikrein (EC 3.4.21.8) releases bradykinin from kininogen by limited proteolysis. The response of the isolated smooth muscle to kallikrein has been reported to be dependent on kinin formation and a subsequent peptide–receptor interaction (Fritz *et al.* [1], Barabé *et al.* [2] and Odya and Goodfriend [3]). However, Beraldo *et al.* [4] reported that rat glandular kallikrein induces rat uterine contractions in the absence of added kininogen. The authors suggested that these uterine contractions were the result of a direct combination of kallikrein with receptors in the muscle. Later Nustad and Pierce [5] reported that repeated additions of rat urinary kallikrein to the isolated rat uterus cause a slow reduction of the response, which is abolished completely after 8–10 hr, but that no change in the response to bradykinin is observed. Nustad and Pierce [5] suggested that the contractile response of the uterus to glandular kallikrein depends on kinin liberation from the kininogen substrate present in the tissue. Beraldo *et al.* [6] demonstrated the presence of kininogen in the rat uterus but concluded that this kininogen was not necessarily related to kinin liberation by kallikrein during the uterine contraction. In addition, Chao *et al.* [7] reported that rat glandular kallikrein can contract an isolated rat uterus without detectable kinin liberation and concluded that the mechanism of kallikrein-induced uterine contraction was uncertain. Recently, Andrade *et al.* [8] reported that the addition of rat plasma kallikrein to the isolated rat uterus elicits desensitization without modification of the response to trypsin. They concluded from these observations that bradykinin is not involved in the contraction elicited by kallikrein and trypsin. To study further the mechanism of kallikrein-induced rat uterus contraction, we used rat submandibular gland kallikrein and concluded that the rat uterine contraction elicited by kallikrein was dependent on kinin formation.

MATERIALS AND METHODS

Rat submandibular gland kallikrein (RSGK). Kallikrein from the submandibular glands of female Wistar rats (180–200 g) was purified and shown to be homogeneous when analyzed by polyacrylamide gel electrophoresis according to Laemmli [9] and by isoelectric focusing according to Figueiredo *et al.* [10].

Kallikrein assay. The contractions induced in the isolated rat uterus by kallikrein were assayed. Virgin Wistar rats (120–140 g) were ovariectomized, allowed to recover for 2 weeks, and injected intramuscularly with Primogyna Depot (Berlimed-Schering, AG) at doses of 5 mg/kg 48 hr before they were killed. The uterine horns were removed, freed from the mesentery, and one horn was suspended in a double-walled bath containing 3 mL of Tyrode's solution at 37° with aeration. Isotonic contractions were recorded with a frontal writing lever. After 1 hr of rest, doses of RSGK were added to the uterus, left in contact with the preparation for 2 min, and then washed out. The assay was repeated at 3-min intervals. In another assay, pure human urinary kallikrein (100 ng) was added to the isolated rat uterus.

Inhibition studies. Trasylol (Bayer, Brazil) (600 KIU) was added to the organ bath containing the uterine horn followed by kallikrein or bradykinin 1 min later. After 2 min of contact, the preparation was washed out. A "Mandevilla vellutina" extract

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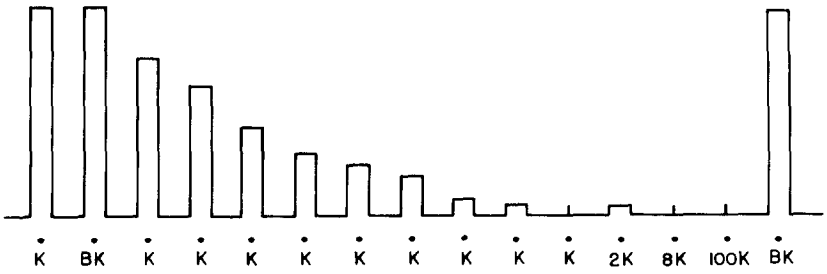

Fig. 1. Copy of a kymographic tracing of an isolated rat uterine contraction. K = 350 ng of rat submandibular gland kallikrein; and BK = 4 ng of bradykinin. (N = 10.)

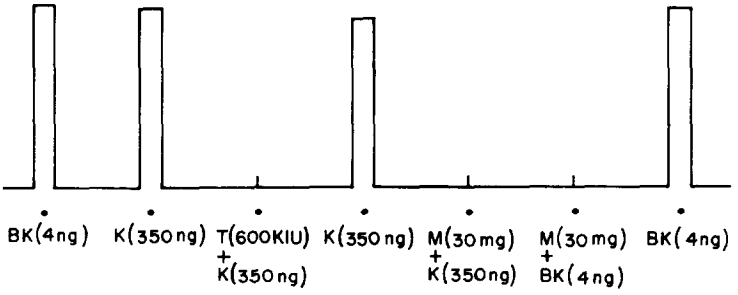

Fig. 2. Copy of a kymographic tracing of the effects of Trasylol and "Mandevilla vellutina" extract on uterine contractions evoked by rat submandibular gland kallikrein and bradykinin. BK = bradykinin; K = rat submandibular gland kallikrein; T = Trasylol; and M = "Mandevilla vellutina" extract. (N = 5.)

(30 mg) (a gift from Dr J. B. Calixto) was added to the preparation, and kallikrein or bradykinin was also added 1 min later. After 2 min of contact, the preparation was washed out.

Rat submandibular gland bradykinin potentiating factor (SBPF). Bradykinin potentiating factor was obtained by the method of Santos *et al.* [11].

Potentiation of the oxytocic effect of kallikrein and bradykinin by rat SBPF. A kallikrein dose eliciting a small contraction of the isolated uterus was chosen. The potentiator was then added to the organ bath, followed by the enzyme 1 min later. The contraction was obtained and the preparation was washed out. The potentiation of the contraction evoked by bradykinin was also assayed by adding SBPF to the preparation, followed by the addition of bradykinin 1 min later. After that, the uterus was depleted of kininogen. The potentiator was then added to the organ bath, followed by the enzyme 1 min later.

Uterine kininogen. Forty uterine horns were removed from virgin Wistar rats (180–200 g) as already described. Twenty horns were used for preparation of the extract and the other twenty were first depleted of kininogen. For this purpose each uterus was suspended in the organ bath containing Tyrode's solution, and doses of kallikrein were added to the bath. The enzyme was left in contact with the preparation for 2 min and then washed out. The same amount of enzyme was added until desensitization of the muscle occurred. Then, larger doses of kallikrein were also added to the preparation in order to obtain a complete desensitization of the uterine horn. After desensitization (kininogen depletion), the uterine horns were removed from the bath, kept in ice-cold Tyrode, and used for extract preparation. Horns were minced and homogenized in 8 mL of ice-cold saline in a Tri R Stir-R homogenizer for 60 sec. The homogenates were centrifuged at 18,000 *g* for 100 min at 4°, and the supernatant fractions were dialyzed and lyophilized.

Kinin release from uterine extracts. The assay for kinin release from uterine extract was performed according to Prado *et al.* [12]. The uterus used for the assay was first depleted of kininogen. After that, the uterine extract was added to the bath, followed by the enzyme 1 min later. The enzyme and the extract remained in contact with the preparation for 2 min, and then the preparation was washed out.

In a similar assay, 500 μL of heated rat plasma (10 mg protein) containing 1 mM Na_2-EDTA was added to the organ bath, followed by 100 ng of human urinary kallikrein 1 min later. After 2 min of contact, the preparation was washed out.

Kinin release from uterine horns. The uterine horn was suspended in a 3-mL bath and left to rest for 1 hr. The uterus was then depleted of kininogen. This preparation was used to detect kinin release from four non-depleted horns also placed in the bath in order to supply kininogen. Kallikrein was then added to the preparation. After 2 min of contact, the preparation was washed out.

Protein determination. Protein concentration was measured by the spectrophotometric procedure of Lowry *et al.* [13] with bovine serum albumin as the standard.

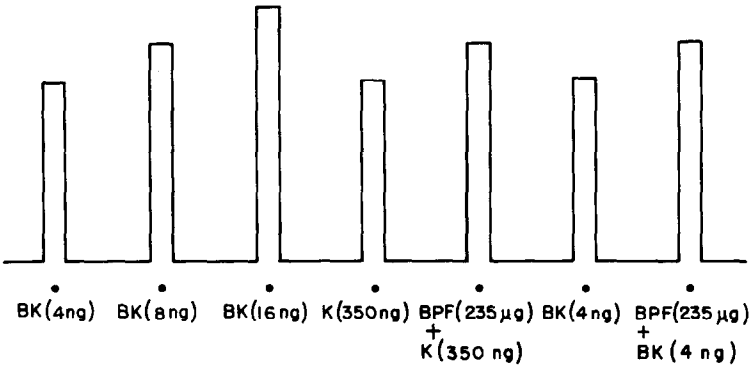

Fig. 3. Copy of a kymographic tracing of the effect of bradykinin potentiating factor on kallikrein and bradykinin-induced contractions of isolated rat uterus. BK = bradykinin; K = rat submandibular gland kallikrein; and BPF = rat submandibular gland bradykinin potentiating factor. (N = 5.)

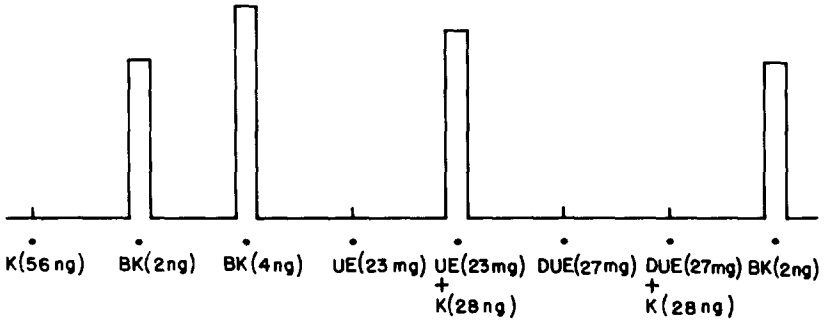

Fig. 4. Copy of a kymographic tracing of the effect of rat submandibular gland kallikrein on rat uterine extracts. K = rat submandibular gland kallikrein; BK = bradykinin; UE = rat uterine extract; and DUE = kininogen-depleted uterine extract. (N = 5.)

RESULTS

The disappearance of kallikrein-induced rat uterine contractions after successive additions of several doses of the enzyme to the organ bath is shown in Fig. 1. There was a gradual decrease of contraction height after several additions of the same dose of kallikrein. Finally, the preparation did not respond even when the enzyme dose was increased 100 times. The figure also shows that 350 ng kallikrein produced a contraction equivalent to 4.0 ± 0.3 ng bradykinin. Human urinary kallikrein (100 ng) applied to an isolated rat uterus did not elicit contraction of the uterus (data not shown).

The inhibitory effects of Trasylol and "Mandevilla velluntina" extract on uterine contraction elicited by kallikrein are shown in Fig. 2. The figure also shows the "Mandevilla vellutina" inhibition of the contraction elicited by bradykinin.

The potentiation of the oxytocic actions of kallikrein and bradykinin by rat glandular bradykinin potentiating factor is shown in Fig. 3. Bradykinin potentiating factor amplified kallikrein and bradykinin-induced uterine contractions to the same extent. After the kininogen depletion, there was no potentiation of the oxytocic action of kallikrein (data not shown).

The role of endogenous kininogen in the contractile response of the uterus to kallikrein is shown in Fig. 4. When 23 mg of rat uterine extract (control) and 28 ng of kallikrein were added simultaneously to the bath, a contraction of the uterus equivalent to 3.0 ± 0.12 ng of bradykinin was observed. However, no contraction occurred when 27 mg (protein) of extract prepared from kininogen-depleted rat uterus was used. Human urinary kallikrein (100 ng) applied to the organ bath containing an isolated rat uterus and 500 μL (20 mg protein) of treated rat plasma did not provoke contraction of the uterus (data not shown).

The kinin released by the incubation of kallikrein with rat uterine horns is shown in Fig. 5. When four horns not depleted of kininogen were placed in the organ bath in the presence of a test kininogen-depleted rat uterus and 350 ng of kallikrein was added to the bath, a contraction of the test uterus equivalent to 2.0 ± 0.01 ng of bradykinin was observed.

DISCUSSION

The present experiments demonstrated that kallikrein-induced rat uterine contraction is dependent

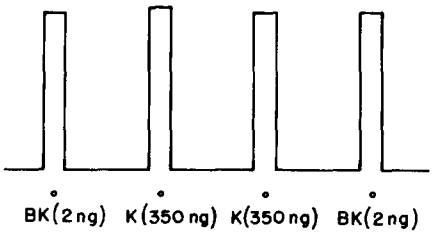

Fig. 5. Copy of a kymographic tracing of the release of kinin from suspended rat uterus by glandular kallikrein. The kininogen was supplied by four uterine horns suspended in the organ bath close to the kininogen-depleted uterus used to assay kinin release. BK = bradykinin; and K = rat submandibular gland kallikrein. (N = 8.)

on kinin formation. This conclusion is supported by the following evidence. When a dose of kallikrein was repeatedly added to the isolated rat uterus, desensitization was obtained (Fig. 1). The desensitization persisted even when the enzyme dose was increased 100 times.

The present results support those published by Nustad and Pierce [5] and by Andrade *et al.* [8]. Our results show that human urinary kallikrein did not release kinin from rat plasma and did not elicit rat uterine contraction when applied directly to the isolated rat uterus, suggesting that the uterine contraction elicited by RSGK is mediated by kinin released from uterine kininogen.

Kallikrein-induced uterine contractions were inhibited by Trasylol and "Mandevilla vellutina" extract. Trasylol is an inhibitor of the enzymatic activity of kallikrein [14], whereas "Mandevilla vellutina" extract contains a substance which is a bradykinin antagonist [15]. This result shows that the contraction was induced by kallikrein and not by some other enzyme possibly present in the kallikrein preparation. Furthermore, the inhibition of kallikrein-induced uterine contraction caused by "Mandevilla vellutina" extract suggests that the oxytocic action of kallikrein is due to bradykinin released from uterine kininogen.

The fact that uterine contraction elicited by kallikrein was potentiated by SBPF (Fig. 3) also suggests that kallikrein releases kinin from uterine kininogen and that the kinin thus released is potentiated. The impossibility of releasing kinin from uterine extract prepared from kininogen-depleted uterus (Fig. 4) is additional evidence that the oxytocic action of kallikrein depends on kinin formation.

The presence of kininogen in the rat uterus was first demonstrated by Beraldo *et al.* [6] who stated that this kininogen is not necessarily related to kinin liberation by kallikrein during the uterine contraction. Chao *et al.* [7] also demonstrated the presence of kininogen in rat uterine homogenates.

In this study we have also demonstrated that kininogen is present in rat uterus (Fig. 4) and that when uterine extract (23 mg in protein) was used as substrate 3.0 ± 0.12 ng of bradykinin was released by 28 ng of kallikrein.

Finally, the contraction of a kininogen-depleted isolated rat uterus elicited by kallikrein acting on four uterine horns used as source of kininogen (Fig. 5) provided elegant evidence that the uterine contraction induced by kallikrein is mediated by the release of kinin from kininogen present in the uterus.

The apparent discrepancy between the present results and those reported by Chao *et al.* [7] may be due to differences in experimental conditions. These investigators incubated 2-cm uterine strips with rat urinary kallikrein in a 10-ml bath and reported that no detectable kinin was present in the incubated medium. However, they admitted that rat glandular kallikrein possibly released kinin from some endogenous uterine kininogen in quantities too small to be detected by radioimmunoassay. In our study we were able to demonstrate the release of kinin by rat glandular kallikrein from endogenous kininogen, using a bioassay.

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