Kallikrein-induced uterine contraction independent of kinin formation

(serine proteinases/smooth muscle/kininogen/kininogenases/kinin antibodies)

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ABSTRACT Responses of smooth muscle to kallikreins (EC 3.4.21.8) are generally considered to result from kinin formation. This premise was reexamined with the isolated rat uterus. Rat urinary kallikrein or bradykinin produced dose-dependent contractions of rat uterus but kallikrein was 5-fold more potent than bradykinin. Kallikrein caused an immediate series of rhythmic contractions which could be increased gradually with subsequent addition of kininogen substrate. Kallikrein-induced contractions were unaffected by carboxypeptidase B or a bradykinin antiserum whereas bradykinin-induced contractions were attenuated or abolished. Other serine proteinases, including trypsin, either did not induce contraction in the absence of added kininogen or did so minimally. Although small amounts of kininogen-like substrate were found in uterine tissue, detectable kinin levels (>4 pg) could not be found in bathing media during maximal kallikrein-induced contractions or after uterine tissue was incubated with high concentrations of the enzyme in the presence of SQ 20851, a kinasase II inhibitor. The data suggest that uterine contraction produced by a homologous kallikrein does not involve kinin formation but results from an action of this serine proteinase upon other accessible systems coupled to the contractile response.

Glandular kallikrein (EC 3.4.21.8) liberates lysylbradykinin (kallidin) from kininogen substrates by limited proteolysis. Although this serine proteinase can attack proinsulin or prorenin in vitro (1, 2), responses of smooth muscle to this kallikrein are attributed to kinin formation and a subsequent peptide–receptor interaction (3–5). However, Beraldo (6, 7) reported that rat glandular kallikrein produced rat uterine contractions in the absence of added kininogen or after pretreatment with actinomycin D or puromycin. It was suggested, but not shown, that these agents might have inhibited endogenous kininogen synthesis, and thus the effects of kallikrein were “direct.” Conversely, the uterine contractile response to glandular kallikrein was suggested to depend on a detected kininogen substrate in the tissue (8, 9).

In the present study we have found that rat glandular kallikrein can cause contraction of the rat uterus without detectable kinin liberation.

MATERIALS AND METHODS

Materials. Rat urinary kallikrein B was purified to homogeneity with ammonium sulfate fractionation and chromatography on DEAE-cellulose, CM-cellulose, and Sephadex G-100. “Vertical slab” polyacrylamide gel electrophoresis produced a single band after Coomassie blue staining. The preparation was also homogeneous when analyzed by polyacrylamide isoelectric focusing (10). An antiserum to pure kallikrein B was raised in a sheep (10). Human urinary kallikrein was purified to homo-

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kallikreins was measured by kinin radioimmunoassay of bath media during contractile responses or after uterine strips were incubated with supramaximal enzyme concentrations in the presence of kininase II inhibition (SQ 20881, 100 μg/ml).

RESULTS

The typical and prompt dose-dependent contractile responses of the rat uterus to kallikrein or bradykinin are shown in Fig. 1 (Upper). The cumulative dose–response curves to kallikrein and bradykinin at concentrations ranging from $9.0 \times 10^{-11}$ to $1.4 \times 10^{-8}$ M. Calculated molar concentrations of kallikrein required to produce 1.0- and 1.5-g increases in contractile force were one-fourth and one-sixth the concentrations of bradykinin required to produce equivalent responses. Kallikrein typically produced rapid and rhythmic contractions followed by spontaneous and full relaxation (Fig. 2). Subsequent addition of kininogen resulted in more rapid contractile responses of augmented force with incomplete relaxation. Kininogen alone never induced uterine contraction (data not shown). Because the kallikrein preparation used for the studies was free of Mg²⁺, the observed rhythmic contractions were induced by kallikrein and not by contaminating magnesium.

Carboxypeptidase B [peptidyl-L-lysine (L-arginine) hydrolase, EC. 3.4.17.2], which degrades kinins, did not alter contractile responses to kallikrein but abolished those to bradykinin (Fig. 3). Bradykinin responses were restored after removal of carboxypeptidase B.

A bradykinin antiserum (1:20 to 1:20,000 dilution) was incubated with either bradykinin (0.2 μM) or kallikrein (60 μM) at 37°C for 1 hr prior to addition of 0.1-ml aliquots to the 10-ml organ bath. There was a dose-dependent inhibition of contractile responses to bradykinin by antiserum, with a 50% reduction of response at a 1:2000 antiserum dilution (final bath dilution, 1:200,000) (data not shown). Responses to kallikrein were never affected by preincubation with bradykinin antiserum. Preimmune serum had no effect on uterine contractions produced by either bradykinin or kallikrein. Kallikrein-induced contractions were not affected by bradykinin antiserum added to the organ bath, but bradykinin-induced contractions were significantly reduced (Fig. 4). Bradykinin responses were restored after removal of antiserum.

Responses to other serine proteinases known to liberate kinins from kininogen substrates were studied. Human urinary kallikrein (11), rat urinary esterase A (12), and trypsin produced the typical uterine contractions when kininogen was preincubated with either proteinase (data not shown). Carboxypeptidase B abolished bradykinin-induced contractions produced by either proteinase (data not shown).
which hydrolyzes kinins
heattreated, alone either
26.5 units/mg, respectively. Similar results were observed with heated and boiled
tissue extracts. Thus, the rat kallikrein (and other kininog-
ases) at supramaximal concentrations with respect to contractile
activity released <10% of the kinin released by trypsin.
Free kinin was not found in uterine extracts. The de Jalon's
solution surrounding isolated rat uteri (n = 3), producing 2.5
g of contractile force in response to kallikrein (4 nM), was with-
drawn and kinin content was measured by radioimmunoassay.
No detectable kinin levels (<4 pg per assay tube) were found
in 0.1-ml aliquots of the media; recovery of added kinin was
98%. Finally, 2-cm uterine strips were incubated in de Jalon's
solution with rat urinary kallikrein (0.5-4 nM) in the absence
or presence of the kininase II inhibitor SQ 20881 (100 µg/ml)
for periods of 10, 30, and 60 min. No detectable kinin was pres-
ent in the incubated media.

**DISCUSSION**

The present study shows that rat uterine contraction produced
by a rat glandular kallikrein occurs without detectable kinin
release. This conclusion is supported by the following findings.
First, the enzyme alone produced prompt contractions not
typical of those which result from a time-dependent initial rate
of enzymatic action and product formation. The enzyme is 4-
to 6-fold more potent than the product bradykinin, used for
comparison as bradykinin is 1.6-fold more potent that kallidin
in the isolated rat uterus (19).
Second, the addition of a kininogen substrate to the organ
bath after kallikrein has initiated maximal and rhythmic re-
sponses produced a time-dependent further increase in con-
tractility, as would be expected secondary to product formation.
Third, both carboxypeptidase B (a nonspecific kininase)
and a specific kinin antiserum abolished the uterine contractile
responses to bradykinin but did not affect those to kallikrein.
Fourth, other potent kininogenases, including human uri-
nary kallikrein (11), rat urinary esterase A (12), and trypsin,
in amounts much larger than those of the rat enzyme either did
not induce uterine contraction or did so minimally.
Fifth, there was no relationship between the calculated
amount of uterine kininogen detected after enzymatic treat-
ment and the ability of the enzymes to induce contractile
responses. That is, amounts of rat urinary kallikrein supramaximal
in terms of contractile responses released <10% of the kinin
from uterine extracts that was released by trypsin; the latter
produced contractile responses <10% of those of kallikrein in
concentrations up to 50-fold higher.
Finally, kinin could not be detected with a sensitive radioim-
munoassay while uteri were contracting in response to kallikrein
or after prolonged incubation of uteri with excess amounts of
enzymes in the presence of kininase II inhibition.
It is known that other rat glandular kallikreins (e.g., pan-
creatic and salivary) cause the isolated rat uterus to contract (20,
21); these enzymes have been considered to be 500-fold more
potent than trypsin insofar as this effect is concerned (22). How-
ever, because small but detectable quantities of a kinin-releas-
ing substrate have been found within the organ (23), the mech-
anism of kallikrein-induced uterine contraction has been
uncertain. The possibility exists that this detectable kininogen
is residual from plasma which contains 3-10 µg of bradykinin
equivalents/ml. On the other hand, the possibility exists that
rat glandular kallikrein uniquely releases kinin from some en-
dogenous uterine kininogen to a location not accessible to avail-
able kinin antibody or kininase and in quantities too small to
be detected by radioimmunoassay. This seems unlikely because
the efficiency of trypsin as a kininogenase is not paralleled by
contractile efficacy.

![Fig. 4. Effect of bradykinin antiserum on kallikrein- and brady-
kinin-induced contraction in the rat uterus. (Upper) Typical tracings
of contractions produced by kallikrein (K) or bradykinin (BK) before,
during, and after addition of bradykinin antiserum (BK-Ab) (1:2000,
final bath dilution). Uteri were washed with fresh medium as soon as
the contractile response reached its peak. (Lower) Contractile re-
sponses as percentage of control before (Ⅳ), during (Ⅲ), and after
(Ⅱ) addition of the antiserum (n = 6).](image)

**Table 1. Effect of kininogenases on rat uterine contraction**

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Bath concentration</th>
<th>% of max contraction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat urinary kallikrein</td>
<td>6</td>
<td>100</td>
</tr>
<tr>
<td>Human urinary kallikrein</td>
<td>60</td>
<td>0</td>
</tr>
<tr>
<td>Rat urinary esterase A</td>
<td>60</td>
<td>0</td>
</tr>
<tr>
<td>Trypsin</td>
<td>30-300</td>
<td>&lt;10</td>
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</tbody>
</table>

Measurements were repeated at least three times in each case. The
specific activities of rat urinary kallikrein, human urinary kallikrein,
and rat urinary esterase A were 125, 74, and 550 Tos-Arg-OMe esterase
units/mg, respectively. One unit is defined as that amount of enzyme
which hydrolyzes 1.0 µmol of Tos-Arg-OMe per min at pH 8.0 and 30°C
in a standard titrimetric assay (17).
Fiedler (24) has pointed out that, although kallikreins are defined by one function (i.e., the ability to liberate a kinin from kininogen), definitive proof that this is the only role of these enzymes is lacking. This is especially pertinent because activities of these serine proteinases not related to kinin are now being discovered. Thus, the abilities of plasma or glandular kallikreins to cleave proinsulin (1), activate prorenin (2), release renin (25), attack angiotensinogen (26), and, now, contract the uterus suggest that the enzymes have other important biologic functions.

It is now known that glandular kallikrein exists in rat plasma (27). Aprotinin, an effective inhibitor of glandular kallikrein, has been found to decrease pregnant uterine muscular activity and prolong the duration of parturition (28). Whether the enzyme has some role in parturition—for example, via the adenyl cyclase activation that occurs in response to various serine proteinases in the rat ovary (29)—remains to be determined.

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