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Does insulin release kinins in rats?

Jacques Damas [a,*], Nancy Garbacki [a], Pierre J. Lefèbvre [b]

[a] Department of Human Physiology, University of Liège, Avenue de l'Hôpital 3, Sart-Tilman, B-4000 Liège, Belgium
[b] Department of Medicine, Division of Diabetes, Nutrition and Metabolic disorders, University of Liège, Avenue de l'Hôpital 3, Sart-Tilman, B-4000 Liège, Belgium

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Abstract

Rat uterus maintained in situ was used as a bioassay of kinins possibly released in vivo by hyperglycaemia or insulin. Intravenous injections of bradykinin induced contractions of rat uterus which were suppressed by HOE 140, a bradykinin B_2 receptor antagonist. Des-Arg^9-bradykinin, a kinin B_1 receptor agonist, did not elicit any response. After propranolol, the effects of bradykinin were enhanced and dose-dependent. This potentiation did not appear in adrenalectomized rats. Captopril, an angiotensin-converting enzyme (ACE) inhibitor, largely increased the effects of bradykinin. In animals pretreated with propranolol, captopril and atosiban, an oxytocin antagonist, intravenous infusion of glucose induced hyperglycaemia and after a delay increased the uterine contractile activity. This contractile effect of glucose was abolished by HOE 140. Infusion of insulin with glucose induced contractions of the uterus. These responses did not appear or were suppressed by HOE 140 or by soya bean trypsin inhibitor (SBTI), a plasma kallikrein inhibitor. These results are direct evidence that insulin induces a release of kinins.

Keywords: Kinin; Bradykinin; Insulin; Glucose; Rat uterus; Angiotensin-converting enzyme inhibitor

1. Introduction

Angiotensin-converting enzyme (ACE) inhibitors improve insulin sensitivity in humans and animals. This improvement mainly results from a reduction of kinin degradation as the influence of ACE inhibitors is largely suppressed by kinin antagonists or by the lack of kininogens from which kinins are released (Review in: Damas et al., 2004). Indeed, ACE is also known to be a kinin degrading enzyme, kininase II (Bhoola et al., 1992). Bradykinin increases glucose uptake and induces glucose-transporter-4 translocation. Bradykinin can thus facilitate several effects of insulin (Review in: Couture and Girolami, 2004). However, other interactions can link insulin to the kallikrein–kinin system. According to Rothschild et al. (1996), insulin infusion in human volunteers reduces plasma levels of high molecular weight kininogen. Furthermore, in vitro incubation of insulin with rat blood results in a decrease in high molecular weight kininogen and plasma prekallikrein associated with an increase in the blood level of a metabolite of bradykinin (Rothschild et al., 1999). These results suggest that insulin might be able to activate the formation of kinins.

To confirm and to examine the extent and time-course of the kinin-releasing effect of insulin, we have measured the formation of kinins induced by glucose and insulin in rats. During a study of potential activators of K_{ATP} channels as relaxant drugs on smooth muscles, we had to develop a test for these substances acting on rat uterus in vivo (Somers et al., 2001). This reminded us that rat uterus in vitro has been largely used to quantify kinin levels in different conditions (Trautschold, 1970). This organ maintained in situ might detect kinins released in vivo. Beside this bioassay for kinins, several immunological methods have been developed to quantify kinin levels (Blais et al., 2000). However, relative large volumes of blood were needed to detect kinins with these methods and only few blood samples can be obtained in a small animal. Thus, only one time assay can be established. Moreover, kinins are locally acting hormones and blood plasma levels do not necessary reflect tissue levels (Vane, 1969; Blais et al., 2000). Thus, a bioassay with rat uterus used in vivo might bring some useful information about

* Corresponding author. Tel.: +32 4 366 5924; fax: +32 4 366 2923.
E-mail address: fa006925@skynet.be (J. Damas).

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the overall time-course of a kinin formation occurring in blood and tissues. Some of our results were presented in a short communication and published in an abstract form (Damas and Garbacki, 2003).

2. Materials and methods

2.1. Animals

Experiments were performed on female Wistar rats (mean weight about 220 g) bred at the animal facilities of the Medical Faculty of the University of Liège. The animals were housed at 22 °C with a 12 h light/dark cycle and free access to food and drinking water. However, some of them were used after an overnight fast. All experiments were carried out in accordance with the guidelines established by our local Institutional Animal Welfare Committee.

2.2. Surgical techniques

Female rats were injected intramuscularly with 200 μg/kg diethylstilbestrol diproprionate. After 24 to 96 h, they were anaesthetized with sodium pentobarbital (Nembutal, 50 mg/kg) intraperitoneally. A catheter was inserted into a jugular vein for intravenous injections or infusions of bradykinin and the other drugs. The animals were tracheotomized. The abdomen was opened through a midline incision. The right uterine horn was separated from the ovary, the ovarian extremity of the uterus was attached under 2 g of tension to an isometric force transducer and the uterine contractions were recorded. Great care was taken to leave intact uterine blood vessels. The abdominal incision and the intestinal tissues were recovered with soft paper soaked with physiological saline (NaCl 0.9%). In some animals, the adrenals were removed before the separation of the uterine horn from the ovary.

2.3. Uterine responses to bradykinin

After a 15 to 25 min equilibration period during which the uterus was challenged with intravenous injections of bradykinin (2 nmol/kg) to examine the reactivity of the uterus, a dose-response curve for bradykinin (0.2 to 12 or 20 nmol/kg) was recorded. Each bradykinin injection was separated from the previous one by a delay of 120 to 180 s. Thereafter, saline or a drug, propranolol, captopril, enalaprilat, HOE 140 or atosiban, was intravenously administered and after a delay of minimum 5 min, another dose–response curve for bradykinin was obtained. The contractile responses of the uterus were measured in g and quantitated by integrating the area under the force–time curve (AUC) above basal resting tone and expressed in g min (Downing et al., 1989).

2.4. Release of kinins in vivo

In a second set of experiments, the potential kinin-releasing activity of hyperglycaemia or of insulin was investigated. Rat uterus was prepared as above and the right carotid artery was isolated to allow blood withdrawal. After the surgical preparation of the animal, the rat was treated with three sucessive intravenous injections of captopril (2 mg/kg), propranolol (2 mg/kg) and atosiban (1.5 mg/kg), respectively. Previously, we have checked that at this dose, atosiban suppressed the myostimulating effect of oxytocin (5 to 50 μg/kg) and did not modify the effects of bradykinin for at least 2 h. Thereafter, the effect of exogenous bradykinin (0.5 nmol/kg) was recorded until a stable response was obtained. Then, 15 to 20 min after captopril administration, an intravenous infusion of physiological saline (0.25 ml/min) was performed for 15 min in order to record the spontaneous activity of the uterus. This first perfusion was followed by two other periods of 15 min infusion of either physiological saline, glucose or insulin with glucose. In order to identify the factors responsible for the uterine activity recorded during the infusion of glucose or insulin, some animals were treated with HOE 140 (0.75 nmol/kg) or with SBTI (6 mg/kg), a plasma kallikrein inhibitor. The contractile activity of the uterus during these three periods was estimated by the number of contractions observed by min and by integrating the total area under the contractile curve (AUC) above basal tone. The contractile activity of the uterus during the first period of infusion of saline was taken as control 100%. Blood glucose was determined in rats receiving glucose or insulin before and after each infusion using Accu-Chek sensor from Roche (Vilvoorde, Belgium).

2.5. Drugs

Bradykinin acetate, des-Arg^9-bradykinin, diethylstilbestrol, oxytocin and soya bean trypsin inhibitor (SBTI) were obtained from Sigma (Antwerpen, Belgium), propranolol hydrochloride from ICI (Macclesfield, U.K.), enalaprilat from Merck Sharp and Dome (Brussels, Belgium), nembutal from Sanofi (Brussels, Belgium), insulin (Actrapid) from Novo Nordisk (Brussels, Belgium), atosiban (Tractocil) from Ferring S.A. (Aalst, Belgium) and captopril from Squibb (Brussels, Belgium). HOE-140 (D-Arg[Hyp^3,Thy^5,D-Tic^7,Oic^8]bradykinin) was a kind gift of Hoechst AG (Frankfurt, Germany). The drugs were dissolved or diluted in physiological saline except diethylstilbestrol dipropionate which was dissolved in arachis oil.

2.6. Data analysis

Results are expressed as mean±S.E.M. Statistical significance was evaluated using Student's *t*-test or one-way analysis of variance (ANOVA) followed by Fisher's protected least significant difference as appropriate, with Statview Student as statistical software. Differences between means were considered significant when a 2-tailed value of P was less than 0.05.

3. Results

3.1. Stimulating effect of bradykinin

In anaesthetized rats, intravenous injections of bradykinin induced contractile responses (Fig. 1) of rat uterus from the dose

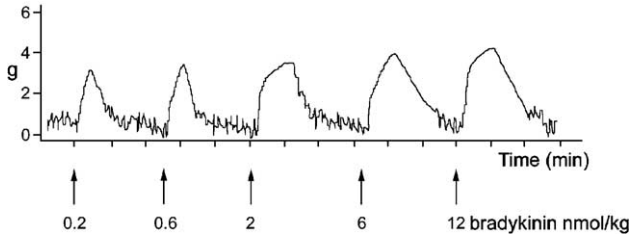

Fig. 1. Effect of bradykinin on uterus of a rat pretreated with propranolol (2 mg/kg). Dose–response curve to bradykinin: contractions of the uterus expressed in g versus time in min.

of 0.2 to 0.6 nmol/kg. The responses to bradykinin were easily differentiated from spontaneous contractions. These responses started 4 to 7 s after the injection and developed faster than spontaneous contractions. They increased with the amount of bradykinin up to a maximum obtained with about 2 nmol/kg and then tended to decrease (Fig. 2A). They were relatively short-lasting 20 to 130 s. No periodic response to bradykinin was observed. These responses to bradykinin were reproducible for at least three consecutive dose–response curves. The stimulating effect of bradykinin was abolished by HOE 140 (0.75 nmol/kg), a bradykinin B_2 receptor antagonist. Des-Arg^9-bradykinin, which stimulates kinin B_1 receptor, administered up to the dose of 750 nmol/kg was without any contracting effect on the uterus. The responses of the uterus to bradykinin depended thus on the stimulation of bradykinin B_2 receptors, as already described in vitro (Birch et al., 1991).

Intravenous injections of bradykinin induce a stimulation of the adrenal medulla resulting in a release of catecholamines (Lecomte et al., 1961) which might reduce the responses of the uterus to the peptide. Indeed, β-adrenergic stimulating drugs, such as salbutamol or fenoterol, are known as tocolytic agents (Abel and Hollingsworth, 1986). After treatment of the rats with propranolol (2 mg/kg), a β-adrenoceptor antagonist, the contractions elicited by the larger doses of bradykinin were significantly increased (Fig. 2A). Bradykinin-induced catecholamine release originates from the adrenals because in adrenalectomized rats, the responses to bradykinin were dose-dependent, largely increased in comparison with the contractions obtained in normal rats and were not modified by propranolol (Fig. 2B).

Bradykinin is rapidly inactivated by several kininases mainly ACE present in rat blood (Dendorfer et al., 2001) and uterus (Fisher and Pennefather, 1997). Therefore, the influence of two different ACE inhibitors on the action of bradykinin was examined. Captopril (9.2 μmol/kg) markedly increased the effect of bradykinin (Fig. 2C). The maximum contraction induced by 12 nmol/kg bradykinin was increased by a factor 4.3 in captopril-treated rats. A similar potentiation was obtained with enalaprilat (0.70 μmol/kg), another ACE inhibitor (data not shown).

The intravenous administration of captopril or of enalaprilat alone elicited transient contractile responses of the uterus. When captopril was intravenously given alone at increasing consecutive doses from 4.6 to 46 μmol/kg, it induced contractile responses of the uterus which did not increased but progressively faded and disappeared after two or three injections made at 5 min interval ($n=6$). The stimulating effect of both ACE inhibitors was not observed in HOE-treated rats ($n=4$) suggesting the involvement of kinins. Therefore, we compared the whole contractile activity of the uterus for 6 to 10 min after saline administration or captopril injection (18.4 μmol/kg) in rats ($n=4$) pretreated with propranolol (2 mg/kg). After saline injection, the mean contractile activity of the uterus was 1.51±0.11 g/min. After injection of captopril alone, the resting tone of the uterus was slightly increased and its overall contractile activity was significantly enhanced to 2.59±0.06 g/min ($P<0.01$). This increase was suppressed by HOE 140 (0.75 nmol/kg): the mean contractile activity was brought back to 1.12±0.14 g/min ($P<0.02$ versus captopril injection).

The responses of the uterus to exogenous bradykinin were thus greatly increased after treatment of the animals with propranolol (2 mg/kg) and captopril (9.2 μmol/kg). To reduce the spontaneous activity of the uterus, rats were also treated with atosiban (1.5 mg/kg), an antagonist of oxytocin, which did not modify the effects of bradykinin. In rats treated successively with these three substances, the threshold dose of bradykinin infused for 5 min was about 0.2 nmol/kg min. At this dose, bradykinin induced periodic contractions of the uterus in 4 rats and was without effect in 2 others. These contractions were of low frequency (0.36±0.13 contraction per min). For higher doses of bradykinin (0.4 and 1 nmol/kg min), all the experiments showed uterine contractile responses to bradykinin infusion and the frequency of phasic contractions increased (respectively, 0.52±0.1 and 0.74±0.06 contraction per min). For 5 nmol/kg min of bradykinin, a large and steady contraction of the uterus occurred during the whole period of infusion (Fig. 3A).

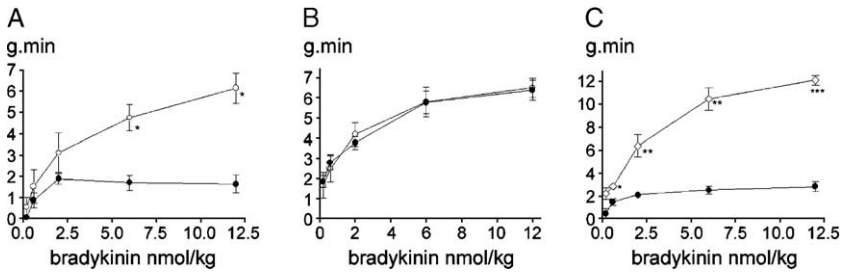

Fig. 2. Dose–response curves to bradykinin in normal rats (a and c) or in adrenalectomized rats (b) after saline (●), propranolol (2 mg/kg;○, a and b) or captopril (9.2 μmol/kg; ◇, c) injections. Data points are mean responses±S.E.M. of 6 determinations and are expressed as AUC in g min. *$P<0.05$; **$P<0.01$, ***$P<0.001$.

3.2. Hyperglycaemia and kinin release

After treatment of the animals with propranolol, captopril and atosiban, the spontaneous contractile activity of the uterus horn was relatively stable for at least 90 min. Indeed, during 3 consecutive periods of 15 min infusion of saline performed in 6 animals, the mean overall activity showed a tendency to increase but not significantly (Fig. 4). Similar results were obtained in 8 other rats submitted to 2 periods of saline infusion (data not shown).

To induce a release of endogenous insulin, glucose was infused in two groups of fasting rats at two different doses, after a control infusion of saline. In the first group (20 mg/kg min, $n=8$), blood glucose increased from 1.03±0.07 g/l after saline infusion to 1.71±0.07 g/l after a first 15 min infusion of glucose and reached 1.92±0.14 g/l after a second period of 15 min infusion of glucose. In the second group (50 mg/kg min, $n=6$), a larger increase in blood glucose levels was observed: blood glucose increased from 0.93±0.04 after saline to 2.80±0.11 g/l ($P<0.001$ versus the first group) after the first 15 min infusion of glucose and to 3.72±0.45 g/l ($P<0.005$ versus the first group) after the second one. During the first period of glucose infusion in both groups, the contractile activity of the uterus did not change significantly (Fig. 4A): it was increased in 2 animals in both groups and reduced in the others. However, during the second period of glucose infusion, the contractile activity increased in 5 animals among 8 in the first group and in all animals in the second one (Fig. 4B). In this second group, this increase started after a delay of 3.7±1.6 min and went on after the end of glucose infusion. The response of the uterus to glucose infusion were equivalent to the responses obtained with infusion of 200 to 600 pmol/kg min of bradykinin (Fig. 3B). In this later group, 15 min after the end of the second period of glucose infusion (50 nmol/kg min), blood glucose level had decreased to 1.73 ±0.45 g/l and at this time, administration of HOE 140 (0.75 nmol/kg) suppressed or largely reduced uterine contractile activity (Fig. 3B) which became similar to that observed during saline infusion (103±8%).

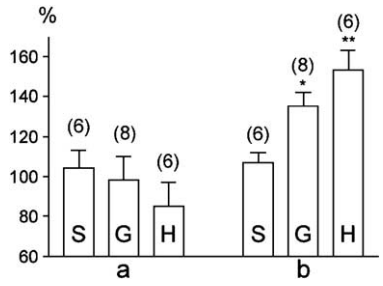

Fig. 4. Mean integral uterine contractile activity/min during two consecutive periods of liquid infusion (a and b): infusion of saline (S), infusion of glucose (20 nmol/kg min, G) or infusion of glucose (50 nmol/kg min, H). The values recorded during a control period of saline infusion were taken as reference values 100%. All the animals have been pretreated with propranolol, captopril and atosiban. Number in parentheses are numbers of observations. *$P<0.05$, **$P<0.01$ versus saline infusion.

3.3. Kinin release induced by insulin

In other animals ($n=8$) treated with propranolol, captopril and atosiban, after a first infusion of saline, two consecutive periods of 15 min infusion of insulin (1 U/kg min) were performed. Insulin administration was associated with that of glucose (20 mg/kg min) in order to keep blood glucose level between 0.9 and 1.2 g/l.

After about 3 min during the first period of insulin infusion, the contractile activity of the uterus increased: either periodic contractions appeared in a previously quiescent uterus or the amplitude and frequency of spontaneous contractions were enhanced. During the second period of insulin infusion, the contractile activity of the uterus stayed at this same level (40±6% increase; Fig. 5). The uterus of two animals was quiescent during saline infusion. In these 2 rats, uterine contractions were observed during the first insulin

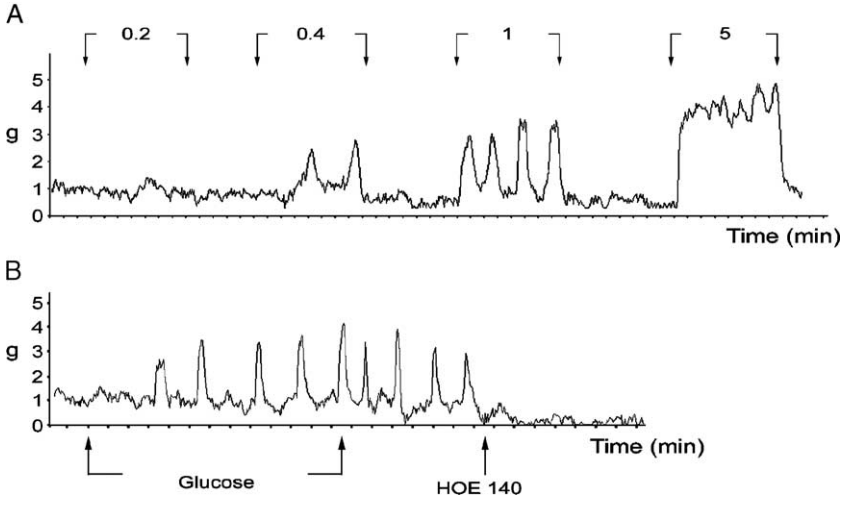

Fig. 3. Contractile activity of the uterus (A) in response to bradykinin infusion (nmol/kg min) for 5 min; (B) during and after the second period of glucose infusion (50 nmol/kg min) in rats pretreated with propranolol, captopril and atosiban. Inhibitory effect of HOE 140 (0.75 mmol/kg). Contractions of the uterus expressed in g versus time in min.

administration. The second period of insulin infusion was then delayed for 10 min. When the first infusion of insulin was stopped, the contractions progressively faded away and then clearly reappeared during the second insulin infusion.

Administration of insulin induced contractions of the uterus. To identify the factor responsible of this increase in contractile activity, rats were treated with HOE 140 (0.75 nmol/kg) either at the start of the experiments before insulin infusion ($n=12$) or between the two periods of insulin infusion ($n=6$). In the first group, insulin infusion did not stimulate the uterus (Fig. 5). In the second group, while the first infusion of insulin increased uterine contractions (Fig. 5A), the contractile activity of the uterus was decreased during the second infusion performed after HOE 140 administration (Fig. 5B). This inhibitory influence of HOE 140 would indicate that kinins were involved in the stimulatory effect of insulin on the uterus.

A major pathway of kinin formation is the activation of plasma prekallikrein which releases bradykinin from high molecular weight kininogen (Bhoola et al., 1992; Blais et al., 2000). To examine the involvement of this enzyme, SBTI, an inhibitor of plasma kallikrein (Vogel and Werle, 1970), was administered between the two periods of insulin infusion, at the dose of 6 mg/kg which reduced the level of kinins in sponge exudates (Damas et al., 1990). Again, in this group of animals ($n=6$), while the contractile activity of the uterus increased during the first insulin infusion (Fig. 5A), this activity returned after SBTI administration to the basal level (Fig. 5B). SBTI, by itself, did not modify the spontaneous activity of the uterus.

In another group of 6 animals treated with propranolol, captopril and atosiban, we performed consecutive infusions of saline, of insulin (1 U/kg min) with a high glucose dose (50 mg/kg min) and then of saline. In this group, blood glucose level did not significantly vary. During infusion of insulin with glucose, the frequency of contractions was not significantly enhanced but the whole contractile activity of the uterus was increased by 106±29% ($P<0.005$) and returned to the basal level during the third infusion period with saline (Fig. 6).

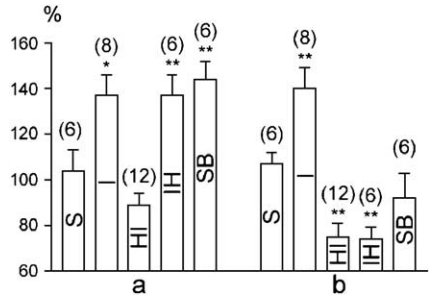

Fig. 5. Mean integral uterine contractions during two consecutive periods of liquid infusion (a and b): infusion of saline (S) or infusion of insulin (1 U/kg min) with glucose (20 nmol/kg min) in rats pretreated with saline (I), in rats pretreated with HOE 140 (HI), or in rats treated with HOE 140 (IH) or with SBTI (SB) between both periods of insulin infusion a and b. The values recorded during a control period of saline infusion are taken as reference values 100%. All the animals have been pretreated with propranolol, captopril and atosiban. Number in parentheses are numbers of observations. *$P<0.05$ versus saline infusion; **$P<0.01$ versus saline infusion.

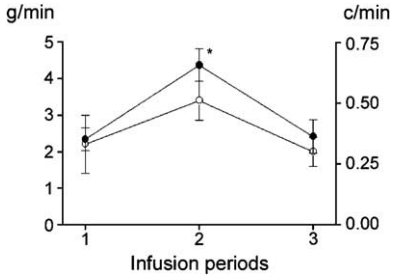

Fig. 6. Increase in contractile activity of the uterus during infusion of insulin (1 U/kg min) with glucose (50 mg/kg min). Mean integral of uterine contractions (g/min; left scale, ●) and mean frequency (contraction/min; c/min, right scale, ○) during three periods of liquid infusion. The numbers of the abscissa indicate the type of infusion: 1 and 3 of saline, 2 of insulin with glucose. All the animals have been pretreated with propranolol, captopril and atosiban. Data points are mean responses±S.E.M. of 6 determinations. *$P<0.005$ versus both saline infusions.

4. Discussion

Rothschild et al. (1999) have reported that insulin infusion for 30 min in rats decreased the plasma levels of plasma prekallikrein and of high molecular weight kininogen and they have suggested that insulin can modulate kinin formation. Assuming that the blood content in rats amounts to 7.5% body weight and that the haematocrit in rats is about 40% (Damas, 1994), this decrease in high molecular weight kininogen would correspond to a release of 100 to 200 pmol/min of bradykinin, according to the values reported by Rothschild et al. (1999). We have tried to detect with our bioassay (Damas and Garbacki, 2003) this possible formation of kinins.

Rat uterine contractions induced by bradykinin is a well-known classical assay for kinins (Trautschold, 1970). In vivo, the effect of bradykinin on uterus of oestrogen-primed rats is largely prevented by a release of catecholamines from the adrenal medulla that the peptide elicits. Indeed, the contractile effect of bradykinin was largely increased by a β-adrenergic antagonist and this potentiating effect was not observed in adrenalectomized animals. This interference of catecholamines could explain why Fregnan and Glässer (1964), who have examined before us the activity of different peptides on uterus in situ of several animal species, have reported that bradykinin induced only《small and evanescent responses》on rat uterus in vivo. Previously, it has been demonstrated that this release of catecholamines modifies the hypotensive (Lecomte et al., 1961) and bronchoconstrictor effect of bradykinin (Collier et al., 1965) and induces an hyperglycaemia in response to intravenous infusion of bradykinin (Damas et al., 2001). The treatment of our animals with a β-adrenergic antagonist thus improves the stimulating effect of kinins on the uterus. Moreover, it reduces the inhibitory influence of adrenaline on the release of insulin from the pancreas (Damas et al., 2001).

To increase the sensitivity of our assay, rats were treated with ACE inhibitors. In rat blood, ACE would be responsible of 52% of the breakdown of bradykinin (Dendorfer et al., 2001) and rat

uterus contains an appreciable activity of ACE (Fisher and Pennefather, 1997). Moreover ACE inhibitors have been reported to own additional degradation-independent actions to increase the effects of bradykinin (see for instances: Dendorfer et al., 2003; Minshall et al., 2000; Tom et al., 2003). Our results show that ACE inhibitors largely potentiate in vivo the stimulating effects of bradykinin on the uterus.

Several observations have suggested that a subthreshold amount of kinins is continuously formed in the cardiovascular system and that in ACE inhibitor-treated animals, this amount would be increased, explaining a part of the hypotensive and cardioprotective effects of these drugs (among others: Arbin et al., 2000; Bao et al., 1992a,b; Baumgarten et al., 1993; Danckwardt et al., 1990; Duncan et al., 2000). However, an appreciable increase in plasma level of kinins after treatment with an ACE inhibitor is rather difficult to display with immunological assays (Blais et al., 2000; Carbonell et al., 1988; Majima et al., 1996). In our animals, ACE inhibitors induced transient contractile responses and increased uterine tone and overall contractile activity of the uterus. These effects were prevented or suppressed by a bradykinin B_2 antagonist. In vitro, ACE-inhibitors do not stimulate by themselves bradykinin B_2 receptors but can develop relaxant or contractile effects in the presence of bradykinin but not of other agonists (Minshall et al., 2000; Tom et al., 2003). Thus, the increase in uterine contractile activity and resting tone that we observed would indicate that the inhibition of ACE and thus of kinin breakdown results in a potentiation of the effects of kinins present and spontaneously formed in the uterus.

To detect a kinin formation following insulin administration, rats were thus treated with propranolol and captopril. Moreover, in order to minimize the spontaneous activity of the uterus, the animals were also treated with atosiban, an oxytocin antagonist. In these conditions, infusion of insulin with glucose to maintain euglycaemia increased the contractile activity of the uterus. This increase did not depend on liquid infusion as saline administration was without significant effect on the uterus. This increase appeared after a short delay during the first infusion of insulin and was relatively stable during the whole period of insulin administration. It disappeared when insulin infusion was stopped. It depended thus on the presence of insulin. This increase was not observed in rats pretreated with HOE 140, a bradykinin B_2 antagonist, and was suppressed by SBTI, a plasma kallikrein inhibitor. It involved thus plasma kallikrein and kinin B_2 receptor. A stimulating effect of tissue kallikrein on rat uterus has been previously described (Nustad and Pierce, 1974). Though tissue kallikrein has been reported to activate directly bradykinin B_2 receptor in vitro (Hecquet et al., 2000), the main stimulating influence of kallikrein acting at physiological concentrations on smooth muscles is mediated through the formation of kinins into the tissue or in the blood (Houle et al., 2003). For instance, the effect of kallikrein on uterus and duodenum withdrawn from normal or kininogen-deficient rats is proportional to the kininogen content of the tissues (Damas et al., 1995). The increase in uterine contractile activity observed during insulin infusion depended thus on the stimulation of B_2 receptors by kinins formed by plasma kallikrein. This increase could be explained by a potentiating influence of insulin towards the effects of kinins. Indeed, insulin has been described to potentiate bradykinin-induced inositol 1,4,5-triphosphate in neonatal rat cardiomyocytes (Kudoh et al., 2002). However, Rothschild et al. (1996, 1999) have observed that during insulin infusion in humans and rats, the plasma level of high molecular weight kininogen decreased while the plasma level of low molecular weight kininogen, which is poorly susceptible to the kinin-forming activity of plasma kallikrein (Bhoola et al., 1992), remained unaffected. All these observations suggest that insulin administration induces an activation of plasma kallikrein which releases bradykinin from its substrate, high molecular weight kininogen. The uterine responses to insulin administration corresponded to the contractions elicited by 200 to 600 pmol/min kg of intravenously injected bradykinin. Taking into account the metabolism of bradykinin by kininases distinct from ACE, such as aminopeptidase P and neutral endopeptidase 24.11 (Blais et al., 2000), our assay agreed with the production of kinins described by Rothschild et al. (1999).

When glucose was administered to anaesthetized rats, the contractile activity of the uterus increased after a long delay when glycaemia was very high. As this increase is abolished by HOE 140, it would also depend on a kinin formation. This formation would follow a release of insulin from the pancreas. The long delay and the high level of blood glucose necessary to obtain a uterine response could be explained by a slow release of insulin. Indeed, catecholamines released by pentobarbital anaesthesia, the surgical procedure and by bradykinin develop an inhibitory influence on the pancreas (Damas et al., 2001). Furthermore, the evolution of blood glucose level during both periods of glucose infusion indicates that the release of insulin in our experimental conditions occurred after a large delay and went on after glucose infusion to bring back blood glucose at a normal level. During this latter time, uterine activity was still increased.

Our assays are thus direct evidence for a modulating role of insulin on kinin formation and indicate that this effect of insulin would be mediated through plasma kallikrein activation. A site candidate for this activation has been recently described at the surface of endothelial cells (Schmaier, 2002). This release of kinins induced by insulin plays a physiological role, as demonstrated by the decrease in insulin sensitivity of kininogen-deficient rats (Damas et al., 1999) and by the facilitating influence of ACE inhibitors towards the metabolic effects of insulin (Damas et al., 2004). However, beside this releasing activity, insulin can also modulate other elements of the kallikrein–kinin system, such as the number of kinin receptors and the kallikrein and kininogen content of tissues and plasma as summarized by Couture and Girolami (2004).

In conclusion, while there is a continuous basal formation of kinins, indicated by the increase in contractile activity of the uterus after ACE inhibitor administration, insulin infusion releases additional amounts of these peptides.

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