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Contractile Responses to Angiotensin I and Angiotensin II in Hamster Uterine Smooth Muscle[1, 2] (39175)

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The lungs are thought to be the major site for enzymatic conversion of angiotensin I to angiotensin II (1–4). However, we have developed evidence suggesting that significant local conversion of angiotensin I (A-I) to angiotensin II (A-II) can occur in the canine renal (5), mesenteric (6), hepatic (7), and hindlimb and coronary vascular beds (8).

Recently, Terragno *et al.* (9) and Ferris *et al.* (10) suggested that A-II may be importantly involved in local regulation of uterine blood flow. Therefore, it becomes important to determine whether or not A-I can be enzymatically converted to A-II by uterine smooth muscle. In this context, Bumpus *et al.* (11) reported that A-I showed only 2 to 3% of the oxytocic effect of A-II on the isolated rat uterus. Similar findings were reported by Needleman *et al.* (12) and Rabito *et al.* (13). The extent to which local conversion of A-I to A-II occurs in uterine preparations from other mammalian species, such as the hamster, is essentially unknown.

The purpose of this study was to examine the effects of A-I and A-II on the isolated hamster uterus in the presence and absence of a compound that inhibits angiotensin-converting enzyme and a compound that blocks A-II receptors. The extent to which contractile responses elicited with A-II are compatible with receptor–occupancy theory for drug action was also tested.

Methods. Adult female golden hamsters (Engles Laboratory, Farmersburg, Ind.) were ovariectomized under pentobarbital (90 mg/kg, ip) anesthesia. The animals were primed by subcutaneous administration of estradiol-17β (15 μg/day) for 3 days following surgery. They were quartered in a controlled environment with a 13:11 photoperiod (lights on 0500–1800 hr) as described previously (14).

Longitudinal strips of uterine smooth muscle measuring about 12 to 14 mm in length and about 2 to 4 mm wide were prepared from each of the two uterine horns immediately after sacrificing the animals with ether. Both strips were mounted in a single muscle chamber according to procedures described by Daniel (15). One end of each strip was fixed to a separate post while its other end was tied to a precalibrated isometric force transducer (Grass, FT-03) with a silk thread. The strips were stretched to a resting tension of 2.5 g and equilibrated for 60 to 90 min in physiological salt solution (20 ml, PSS) maintained at 37° and continuously aerated with 95% O_2 and 5% CO_2. The pH of the PSS was 7.3 to 7.4 and its composition (m*M*) was NaCl, 130; KCl, 4.7; KH_2PO_4, 1.18; $MgSO_4 \cdot 7H_2O$, 1.17; $CaCl_2$, 0.5; $NaHCO_3$, 14.9; and dextrose, 5.5. Low concentrations of $CaCl_2$ were used in order to eliminate spontaneous contractile activity.

Purified synthetic angiotensin I (A-I, Beckman) or angiotensin II (A-II) were dissolved in PSS and added (0.2 ml) to the tissue bath. The contractile response occurring during 3 min of contact was recorded (Grass, P-7). Dose–response curves were constructed with the single dose technique rather than the cumulative dose technique. Responses were evaluated by measuring the area (K & E plainimeter 4236M) circumscribed by the graphic tracing of the con-

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tractile event and expressed as a percentage of the maximal response evoked with A-II. The tissue bath was rinsed five times with fresh PSS, and at least 20 min elapsed between challanges so that tachyphylaxis did not develop. The muscle strips were challenged with a maximal dose of KCl (50 m*M*) at the beginning and end of each experiment. Experiments were discarded when initial and late responses to KCl differed by more than 10%.

Angiotensin-converting enzyme was inhibited by SQ-20881 (Pyr-Trp-Pro-Arg-Pro-Gln-Ile-Pro-Pro) and P-113 (1-Sar-8-Ala-A-II) was used to block A-II receptors (7, 8, 16). Cholinergic blockade was produced with atropine, whereas α-adrenergic blockade was produced with phentolamine. Statistical significance of differences between responses to A-I or A-II in the presence and absence of inhibitors were assessed with Student's t test and required that $P < 0.05$.

A theoretical dose-response curve was constructed for A-II as described by Van Rossum (17) and Rioux *et al.* (18). That is, the ED_{50} for A-II was estimated from the experimental dose-response curve and used as an approximation for K_A, the apparent dissociation constant of the A-II receptor complex. The theoretical curve was calculated by substituting K_A into Clark's equation,

$$\frac{R}{Rm} = \frac{A}{A + K_A}$$

where R is contractile response, Rm is maximal contraction produced, and A is the dose of A-II added to the bath.

The extent to which A-I could be converted to A-II in the isolated uterus of the hamster was deduced from a comparison of experimental dose-response curves for each agonist utilizing standard bioassay procedures (5–8, 19). Briefly, the molar ratio of the dose of A-II to the dose of A-I required to elicit the same increase in isometric tension was taken as a measure of the amount of conversion.

Results. A-I and A-II produced dose-dependent increases in isometric tension that were often accompanied by phasic contractile activity (Figs. 1–3). The responses were rapid in onset (5 to 10 sec), attained maximal levels in about 45 sec, and invariably

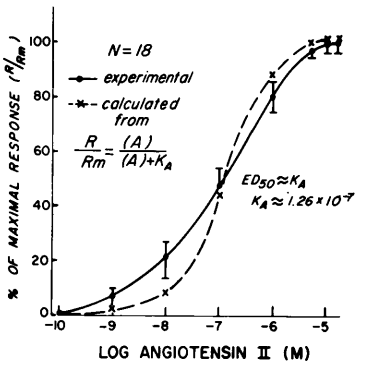

FIG. 1. Experimental and theoretical (calculated) dose-response curves for A-II are shown for uterine smooth muscle isolated from 18 different hamsters. Small vertical bars represent ± 1 SE.

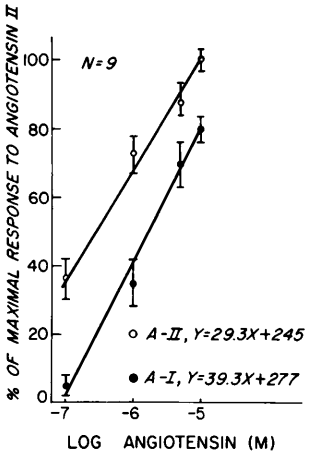

FIG. 2. Effects of graded doses of A-I and A-II, expressed as percentage of maximal response to A-II, are illustrated for nine different uterine preparations. Points represent mean values while small verticle bars represent ± 1 SE. The least-squares line and equation are given for each agonist.

decreased to baseline while either A-I or A-II was still in the tissue bath (Fig. 3).

The concentration of A-II required to elicit a maximal response was about 10^{-5} M, and the ED_{50}, estimated graphically, was about 1.27×10^{-7} M (Fig. 1A). The theoretical dose-response curve generated from Clark's equation and using ED_{50} as an approximation of K_A, the apparent dissociation constant of the A-II–receptor complex, was in close agreement with the experimental dose-response curve (see Methods).

Contractile responses to A-I were

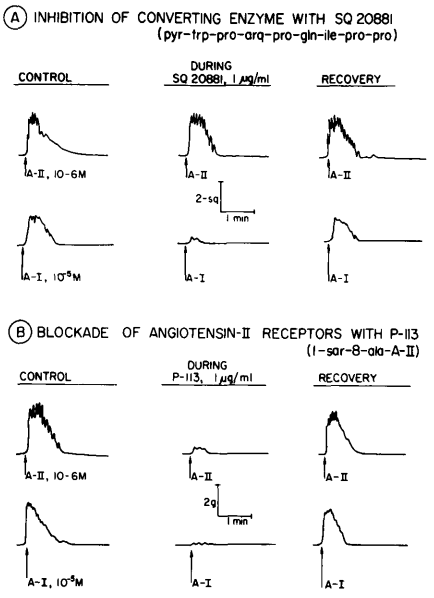

FIG. 3. Effects of A-I and A-II on isometric tension are shown for two different uterine preparations in the presence and absence of SQ20881 (Panel A), and in the presence and absence of P-113 (Panel B). Arrows indicate time of addition of agonist to the tissue bath. Responses in the presence of inhibitors were recorded 10 min after contact with inhibitor. Recovery of responsiveness was examined 30 min after the inhibitor had been washed from the bath.

smaller than responses to equimolar concentrations of A-II throughout the dose range (10^{-7} to 10^{-5} *M*) examined (Fig. 2). For each dose of A-I tested, the corresponding dose of A-II required to produce an equivalent increase in isometric tension can be estimated by inspection of the dose–response curves. For example, 40% of the maximal response to A-II was produced with A-I at a dose of 10^{-6} *M* whereas only 1.4×10^{-7} *M* A-II was required to elicit the same response. If the response to A-I is ascribable to its conversion to A-II, the results suggest that about 14% of the A-I injected into the bath was converted to A-II (see Methods). Similar calculations show that the maximal possible extent to which A-I can be converted to A-II ranges from 14 to 27% (Table I).

Responses to A-I were virtually abolished by SQ20881 (Pyr-Trp-Pro-Arg-Pro-Gln-Ile-Pro-Pro), a potent inhibitor of angiotensin-converting enzyme (Figs. 3 and 4). In contrast, contractile responses to A-II were unaltered by SQ20881. However, P-113 (1-Sar-8-Ala-angiotensin II), an A-II antagonist, inhibited responses to both A-I and A-II. Recovery from the inhibitory effects of either SQ20881 or P-113 occurred within 30 min after the bathing medium was replaced with fresh PSS (Fig. 3).

It is important to note that P-113 did not alter uterine contractions produced with KCl. Thus, the increase in isometric tension produced with KCl (50 m*M*) was 2.54 ± 0.2 g in the presence of P-113 and 2.61 ± 0.3 g in the absence of P-113 (four preparations).

Cholinergic blockade with atropine, or α-adrenergic blockade with phentolamine did not influence responses to A-I or A-II (Fig.

TABLE I. PERCENT CONVERSION OF ANGIOTENSIN I TO ANGIOTENSIN II IN THE ISOLATED UTERUS OF THE ESTROGEN-PRIMED HAMSTER.

Contractile response (% of maximum)	A-I (M)[a]	A-II (M)[a]	Conversion of A-I to A-II (%)
40	10^{-6}	1.4×10^{-7}	14.0
50	1.75×10^{-6}	3×10^{-7}	17.1
60	2.2×10^{-6}	5.8×10^{-7}	26.4
70	5.6×10^{-6}	1.5×10^{-6}	26.8
80	10^{-5}	2.4×10^{-6}	24.0
Mean			21.7 ± 2.5%

[a] Estimated from dose–response curves shown in Fig. 2.

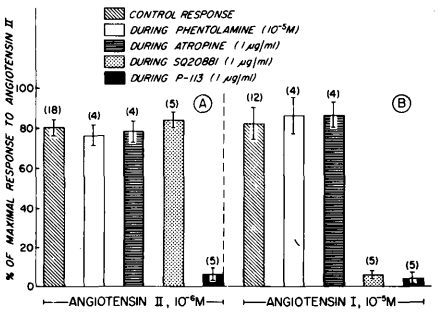

FIG. 4. Influence of α-adrenergic blockade (phentolamine), cholinergic blockade (atropine), inhibition of angiotensin-converting enzyme (SQ20881), and A-II blockade (P-113) on uterine contractile responses produced with A-II (Panel A) or A-I (Panel B). Each bar represents the mean response for the number of strips indicated above it, while the small verticle bars represent ± 1 SE.

4). The dose of atropine was sufficient to block contractile responses to acetylcholine (0.1 to 0.5 μg/ml), and the dose of phentolamine was sufficient to block contractile responses in isolated rat tail arteries with epinephrine (10^{-5} *M*).

Discussion. The present study shows that angiotensin I (A-I) and angiotensin II (A-II) produce dose-dependent increases in isometric tension in uterine smooth muscle isolated from estrogen-primed hamsters (Figs. 1–4). Our findings suggest that responses to A-II are due to its direct interactions with A-II receptors. In addition, responses to A-I appear to be largely due to its local conversion to A-II and subsequent interactions with A-II receptors.

Uterine contractile responses elicited with A-II are quantitatively consistent with receptor occupancy theory based on the law of mass action (17, 18). This conclusion is supported by the findings that experimental and calculated dose–response curves for A-II were in close agreement (Fig. 1). Rioux *et al.* (18), working with isolated smooth muscle preparations from rabbit aorta and rat stomach also concluded that responses to A-II were mechanistically consistent with receptor occupancy theory.

That uterine responses to A-II are probably due to direct interactions of A-II with specific receptors is also supported by other observations. For example, cholinergic blockade with atropine or α-adrenergic blockade with phentolamine did not influence responses to A-II (Fig. 4). In contrast, 1-Sar-8-Ala-angiotensin II, a well-characterized A-II antagonist (7, 8, 16), specifically blocked responses to A-II (Figs. 3 and 4). Contractile responses elicited with KCl were not altered by 1-Sar-8-Ala-angiotensin II (see Results).

Our suggestion that uterine responses to A-I are largely ascribable to local enzymatic conversion to A-II is based on several findings. First, responses to A-I, like responses to A-II, were unaltered by either cholinergic or α-adrenergic blockade (Fig. 4). Second, responses to either A-I or A-II were virtually abolished during blockade of A-II receptors with 1-Sar-8-Ala-angiotensin-II (Figs. 3 and 4). Third, responses to A-I were nearly eliminated by SQ 20881 (Pyr-Trp-Pro-Arg-Pro-Gln-Ile-Pro-Pro), which is a potent inhibitor of angiotensin-converting enzyme (7, 8). In sharp contrast, SQ 20881 did not alter responses to A-II or KCl (Figs. 3 and 4). An alternative explanation for these findings is the possibility that A-I interacts directly with uterine A-I receptors and that such receptors, unlike A-II receptors, are blocked by both 1-Sar-8-Ala-angiotensin-II and SQ 20881. This possibility, however, does not seem likely because A-I exhibits little or no biological activity in most systems studies (1, 4).

The ability of A-I to produce marked increases in isometric tension in uterine smooth muscle from the hamster contrasts with the poor responsiveness of similar preparations from rats (11–13). This disparity may be partly due to the low level of converting enzyme activity assayed in the uterus of rats (3, 11–13) and other factors including differences in hormonal dominance and differences in responsiveness to A-II.

Whether or not the large extent to which A-I appears to be converted to A-II in the isolated uterus of the hamster is importantly involved in regulation of uterine function during the estrous cycle or pregnancy merits further study.

Summary. Angiotensin I (A-I) and angiotensin II (A-II) produced dose-dependent increases in isometric tension in isolated strips of uterine smooth muscle prepared from ovariectomized golden hamsters treated with estrogen. Responses to A-II were consistent with receptor–occupancy theory of agonist–receptor interactions. Inhibition of angiotensin-converting enzyme virtually abolished responses to A-I but not those to A-II. Blockade of A-II receptors inhibited responses to both A-I and A-II. Cholinergic or α-adrenergic blockade did not alter uterine responses to either A-I or A-II. These findings suggest that contractile responses elicited in the isolated uterus of the hamster are due to its local conversion to A-II and subsequent interactions with specific A-II receptors. Such conversion occurs at least to the extent of 14 to 27%.

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