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Lidocaine decreases the xylazine-evoked contractility in pregnant cows

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ABSTRACT

The objective of this in vitro study was to evaluate and compare the effects of xylazine on basal uterine contractility of bovine pregnant uterine strips and that of lidocaine on xylazine-sensitized bovine pregnant uterine strips, at different stages of pregnancy. Basal contractility was evaluated in an isolated organ bath and the functionality of the strips throughout the experiment was evaluated using a dose of carbachol (10^{-5} M). Uterine motility, expressed with amplitude, frequency of contractions as well as the area under the curve, was recorded in different stages of pregnancy and data were collected at 15-min intervals (5-min before and 5-min after xylazine administration and 5-min after lidocaine addition on the *plateau* contraction induced by xylazine). Uterine motility increased in all the stages of pregnancy after xylazine addition and gradually decreased after treatment with lidocaine. These data suggest that lidocaine might decrease the tonic effect induced by xylazine on bovine pregnant uteri.

1. Introduction

Myometrial contractility is widely known to be mediated by alpha-adrenoceptors and both alpha$_1$- and alpha$_2$-adrenoceptors have been localized in the myometrium, through radioligand binding techniques (Marshall, 1970; O'Donnell et al., 1978; Digges, 1982; Taneike et al., 1999). The density of alpha$_2$-adrenoreceptors in the bovine uterus is higher compared to that of alpha$_1$-adrenoreceptors (Ko et al., 1990; Taneike et al., 1999), since the longitudinal layer possesses more alpha$_2$-adrenoreceptors than the circular one, and in both the layers the receptors are heterogeneously distributed (Taneike et al., 1999). It is generally believed that alpha$_1$- but not alpha$_2$-adrenoceptors mediate myometrial contractility (Marshall, 1970; O'Donnell et al., 1978; Digges, 1982; Taneike et al., 1999). Other reports, however, suggest that alpha$_2$-adrenoceptors modulate uterine contractility, too. For instance, xylazine, an alpha$_2$-adrenoceptor agonist used as analgesic/sedative in veterinary practice, increases uterine electro-myographic activity in pregnant ewes (Pérez et al., 1997), augments intrauterine pressure in cows at oestrus and during diestrus (Minoia and Mitolo-Chieppa, 1976; LeBlanc et al., 1984). Furthermore, this drug was shown to exert a contractile effect on bovine uterine strips excised at oestrus and in diestrus, and this effect was shown to be prevented by alpha$_2$- but not alpha$_1$-adrenoceptor antagonists (Minoia and Mitolo-Chieppa, 1976; LeBlanc et al., 1984). Moreover, xylazine promotes contractility in bovine pregnant uteri, mostly in the last trimester of pregnancy, which results in miscarriages or premature births (Rosenberg et al., 1969; Ahlers, 1970; Treu, 1972; Dart, 1999). Indeed, this molecule selectively activates the uterine postsynaptic alpha$_2$-adrenoreceptor (Pérez et al., 1994), leading to the inhibition of adenylate cyclase, the decrease in cAMP, the increase in intracellular Ca^{2+} through the opening of voltage-dependent channels, leading to an increase in myometrial contractility (Jacobs et al., 1985; Ko et al., 1990; Latek et al., 2012).

Even though several studies show that xylazine affects uterine contractility, no study, to the best of the Author's knowledge has still investigated the effect of xylazine in different stages of pregnancy. This study evaluated the in vitro effect of xylazine on bovine uteri, in different periods of gestation (from 30 to 270 days of pregnancy). Furthermore, since xylazine is often administered to cows in association with local anesthetic agents, such as lidocaine (Lumb and Jones, 1990; Clarke et al., 2014; Rizzo et al., 2015a; Rizzo et al., 2015b). This local anesthetic agent acts through the block of sodium voltage-dependent channels, hyperpolarize the membrane and slows the driving of pulse (Cassutto and Gfeller, 2003; Cook and Blikslager, 2008; Anderson and Edmondson, 2013). To the best of the Author's knowledge this mechanism of action could decrease the contractile tone in agreement with in vitro studies performed on pregnant uteri of rats and women (Willdeck-Lund and Nilsson, 1979; Fauza et al., 1999; Fauza et al., 2003; Wei et al., 2014). With this background, the effects of lidocaine on xylazine-mediated uterine contractility were investigated too.

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2. Materials and methods

2.1. Preparation of uterine strips

Seventy-two pregnant healthy uteri were obtained from cows slaughtered at a local abattoir. Pregnancy has been divided into 9 periods of 30 days each (from 0 to 30 days to 240–270 days) and 8 strips for each stage of pregnancy were considered in our study.

Gestational age (in days) was inferred by *ante* and *post-mortem* examination. As to the first trimester, pregnancy status was *ante-mortem* diagnosed by a clinical examination and B-mode ultrasonography (SonoSite MicroMaxx Bothell WA, USA with a 7.5 MHz linear probe), as summarized by Hughes and Davies (1989), and the bovine genital tract was visually examined to confirm the stage of pregnancy (in days) at *post-mortem* investigation. The stage of pregnancy was obtained measuring the pregnant horn and crown-rump length (Harris et al., 1983).

All the uteri were excised in about 20 ± 10 min after slaughtering. From each uterus, a single circular portion of the middle part (equidistant from the bifurcation and the tubo-uterine junction) of the gravid horn was cut and immediately placed in a flask containing pre-refrigerated Krebs solution (NaCl 113 mM, KCl 4.8 mM, $CaCl_2$ $2H_2O$ 2.2 mM, $MgSO_4$ 1.2 mM, NaH_2PO_4 1.2 mM, $NaHCO_3$ 25 mM, glucose 5.5 mM, sodium-ascorbate 5.5 mM), which was daily prepared. The flask was then transported (15 ± 5 min) to the laboratory in an isolated box. From each circular portion, full-thickness uterine strips (10-mm long and 3-mm wide) were cut between two rows of endometrial caruncles and parallel to the longitudinal muscle fibers.

2.2. Experimental design

The strips were immediately placed in a jacketed organ bath (mod. 4050 Ugo Basile, Milan, Italy) containing 10 ml of Krebs' solution and continuously bubbled with a mixture of 95% O_2 and 5% CO_2. The pH was kept at 7.4, and temperature was maintained at 37 °C. A silk thread was used to attach the myometrial strips to a fixed hook belonging to an isometric force displacement transducer (FORT25; AD Instruments, Castle Hill, NSW, Australia). The contractile activities were recorded using an acquisition software (PowerLab 4/35, AD Instruments). During the first 60 min, the strips were let stabilize in the organ baths without applying tension. Subsequently, the strips were allowed to equilibrate under a constant tension of 2 g for about 30 min. After the equilibration period, a first dose of carbachol (10^{-5} M) (Sigma-Aldrich, Milano, Italy), the esterified form of acetylcholine, which has a selective and prolonged contractile effect was added to a cuvette. This dose, dissolved in Krebs solution, was subsequently removed by wash-out, followed by a resting period of 30 min or more, needed for the strip to return to baseline. Subsequently, a second dose of carbachol (10^{-5} M) was added to the cuvette and its effects were compared to those obtained during the previous administration (Piccinno et al., 2014a).

In the presence of a repeatable response with a deviation ≤20%, calculated by the formula: $(Value_{Maximum} - Value_{minimun} / Value_{Maximum}) * 100$, 100, the experimental protocol was tested: a third identical dose of carbachol was administered again, after *wash out* and after a resting period of 30 min. If this final administration of carbachol was not repeatable with at least one of the previous doses, the strip was discarded from the experiment (Piccinno et al., 2014a).

After the stabilization period, the strips were exposed to a single dose of xylazine (10^{-5} M) (Sigma-Aldrich, Milano, Italy) (this concentration was chosen, since it ensures the maximum in vitro uterine stimulation standing to Ko et al., 1990) and was left in the bath for 5 min, in order to allow the realization of the maximum tonic effect. Afterwards lidocaine (10^{-8} M) (Sigma-Aldrich, Milano, Italy) was added without wash out. The concentration of lidocaine employed was that of the minimum concentration of local anesthetic in vivo administered by Wei et al., 2014. The association xylazine-lidocaine was let act for 5 min, then wash-out was performed. Both drugs were dissolved in ethanol which has no effect on in vitro bovine uterine contractility (Rizzo et al., 2010; Piccinno et al., 2014a).

Afterwards, the registration period was followed by the addition of a last dose of carbachol (10^{-5} M), in order to evaluate the functionality of the strip after the experiment. The response of the strip had to be repeatable (within 20%) compared to that for the previous administration (Piccinno et al., 2014a).

For each strip, amplitude, frequency of contractions and the area under the curve (AUC) were registered and assessed before and after the administration of xylazine and xylazine-lidocaine association. The time interval over which such determinations were made was chosen after observing the effect of xylazine on basal contractility and that of lidocaine on the tonic effect previously induced by the $alpha_2$-agonist.

For each administration, the percentage increases or decreases from baseline (basal vs xylazine and basal vs xylazine-lidocaine association) and from xylazine-induced effect (xylazine vs xylazine-lidocaine association) were evaluated using the following formula: $(T_{Second\ value} - T_{First\ value} / T_{First\ value}) \times 100$ (Piccinno et al., 2014a; Piccinno et al., 2014b).

2.3. Statistical analysis

For motility studies, all values of amplitude, frequency and AUC were expressed as Mean ± SEM and underwent statistical analysis by SPSS® Statistics 19 (IBM®, NY).

Intragroup and intergroup variations as well as the percentage increases or decreases in amplitude and frequency of contractions and AUC (basal vs xylazine, basal vs xylazine-lidocaine association, xylazine vs xylazine-lidocaine association) were tested with one-way ANOVA and post hoc LSD (Least significant difference) test.

A value of $p < 0.01$ was set as significant.

3. Results

Spontaneous uterine contractility was observed in 68 out of 72 strips. Four strips did not show any spontaneous or comparable responses to carbachol (10^{-5} M), thus were discarded.

The representative tracings (Figs. 1–3) show the effects induced by xylazine (10^{-5} M) on basal contractility, and by xylazine + lidocaine on xylazine-mediated contractions, at 30–60, 150–180 and 240–270 days of pregnancy, respectively.

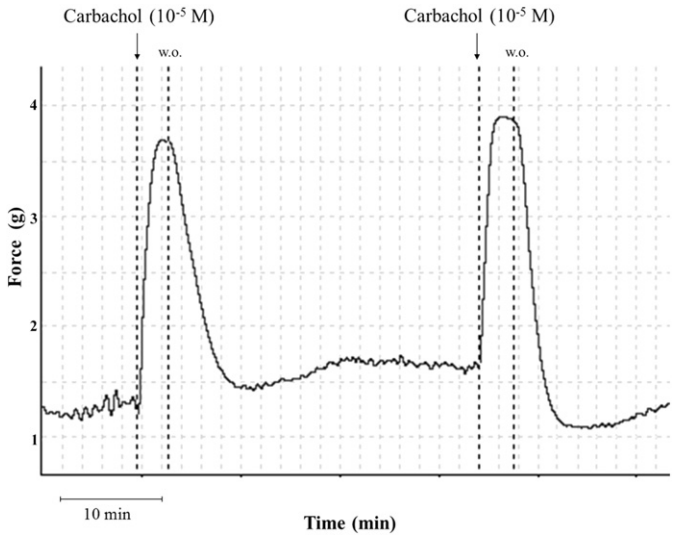

Fig. 1. Representative tracing of the effects induced by xylazine (10^{-5} M) on uterine contractility and by lidocaine (10^{-8} M) on the tonic effect induced by xylazine, at 30–60 days of pregnancy. Amplitude (y axis) is expressed in g.