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Scopolamine butylbromide decreases the xylazine-mediated contractility in bovine pregnant uteri

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ABSTRACT

The objective of this *in vitro* study was to evaluate and compare the effects of scopolamine butylbromide (Spasmolax®) on xylazine-sensitized bovine pregnant uterine strips, at different stages of pregnancy. The procedures were carried out in isolated organ bath. 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 5-min intervals starting 5 min before treatment until 10 min after treatment (5-min after xylazine administration and 5-min after scopolamine butylbromide addition). The results suggest that scopolamine butylbromide might decrease the tonic effect induced by xylazine on bovine pregnant uteri from 0-30 days to 240–270 days.

1. Introduction

Uterine contractility can be modulated by several factors: hormones, such as estrogen, progesterone, oxytocin, prostaglandin$F_{2\alpha}$ [1–3] and neurotransmitters. For example, myometrial contractility is widely known to be mediated by alpha-adrenoceptors (alpha$_1$- and alpha$_2$-adrenoceptors) [4–6] and by binding with muscarinic receptors M2 and M3 [7,8].

The distribution of these receptors depends on the hormonal status: estrogens promote the synthesis of receptors alpha$_2$-adrenergic and muscarinic (M2), while reduce the synthesis of muscarinic receptor M3. Indeed, the high estrogen concentrations occurring at delivery are accompanied by a major density in the alpha$_2$ adrenergic and M2 muscarinic receptor, which contribute to modulate uterine contractility [9,10].

Several *in vivo* and *in vitro* studies showed that xylazine, an alpha$_2$-adrenoceptor agonist used as analgesic/sedative in veterinary practice, increases uterine activity in pregnant cows, not only when estrogen increases (last trimester), but also in the others month of pregnancy, resulting in miscarriages or premature births [6,11–14].

Parasympatholytic drugs or antimuscarinic drugs are substantially competitive muscarinic receptor antagonists, which are often used to control spasms of the smooth musculature, in case of gastrointestinal, uterine, urinary, biliary and bronchiolar hypermotility [15]. Rizzo et al. [8] showed that an antimuscarinic and spasmolytic substance, scopolamine butylbromide (Spasmolax®) might optimize uterine involution and improve pregnancy rates, in dairy farms. This effect could be due to a block of contractions of the uterus in the *postpartum*, for a period of time corresponding to its half-life (2–3 h), By time, the disappearance of the pharmacological effect of scopolamine may awaken uterine involution and the uterus may undergone to physiological and regular postpartum contractility, facilitated by a greater blood flow and with an increase in glandular secretions, useful to the self-cleaning of the uterus itself. Moreover, scopolamine butylbromide induces an increase in the reabsorption of uterine collagen, as evinced by highest levels of hydroxyproline observed in the treated cows.

In this perspective, we investigated the effects of scopolamine butylbromide (Spasmolax®) on xylazine-evoked uterine contractility on bovine uterus, in different stage of pregnancy.

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2. Materials and methods

2.1. Preparation of uterine strips

Eighty-one pregnant healthy uteri were obtained from Holstein Friesian cows, aged 3–5, slaughtered at a local abattoir. Pregnancy was divided into 9 periods of 30 days each (from 0-30 days to 240–270 days) and 9 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 [16], 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 [17].

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 prepared daily. 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 [1,6].

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 allowed to stabilize in 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 [18].

In the presence of a repeatable response with a deviation ≤20%, calculated by the formula: ($Value_{Maximum}$-$Value_{minimun}$/$Value_{Maximum}$)*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 [18].

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 according to Ko et al. [19]) and was left in the bath for 5 min, in order to allow the realization of the maximum tonic effect. Afterwards scopolamine butylbromide (10^{-5} M) (Spasmolax®, ATI, Ozzano Emilia, Italy) was added without wash out. The concentration of scopolamine butylbromide employed was that of the minimum concentration of antimuscarinic drugs *in vivo* administered by Braun et al. [20] and Rizzo et al. [8]. The association xylazine-scopolamine butylbromide was left to 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 [18,21].

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 [18].

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-scopolamine butylbromide association. The time interval over which such determinations were made was chosen after observing the effect of xylazine on basal contractility and that of scopolamine butylbromide 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-scopolamine butylbromide association) and from xylazine-induced effect (xylazine *vs* xylazine-scopolamine butylbromide association) were evaluated using the following formula: $(T_{Second\ value} - T_{First\ value}/T_{First\ value}) \times 100$ [18,22].

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 in amplitude and frequency of contractions and AUC were tested with one-way ANOVA and post hoc LSD (Least significant difference) test.

A value of $p < .05$ was set as significant.

3. Results

Spontaneous uterine contractility was observed in 72 out of 81 strips. Nine strips did not show any spontaneous or comparable responses to carbachol (10^{-5} M) and were therefore discarded.

Amplitude, frequency and area under the curve (AUC) (mean ± SEM) before (basal), after xylazine and scopolamine butylbromide additions in different gestional stages are shown on Tables 1–3.

Amplitude, frequency and AUC of basal contractions showed statistically significant differences among the different stages of pregnancy (Tables 1–6).

Xylazine evoked a tonic effect (amplitude) which gave rise to statistically significant at 30–60 days, 180–210 days and 240–270 days (Table 1).

The addition of scopolamine butylbromide to xylazine-stimulated strips led to a relaxing effect in all the stages of pregnancy, but with different intensity and effectiveness. Indeed, this effect was significant only at 30–60 days, 120–150 days and 240–270 days (Table 1).

Xylazine reduced frequency of contraction in all the stages of pregnancy. This effect was statistically significant, only at 180–210 days and at 210–240 days (Table 2), whereas scopolamine butylbromide always increased the number of contractions per minute, but this rise was significant only at 30–60 days (Table 2).

The area under the curve (AUC) was increased after xylazine and reduced after scopolamine butylbromide. The rise induced by