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Ex vivo influence of carbetocin on equine myometrial muscles and comparison with oxytocin

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Abstract

To determine the intercyclic effect of oxytocin and carbetocin on equine myometrial tissue, the effect of the drugs was evaluated through pharmacokinetic and pharmacodynamic studies. The complete pharmacokinetic profile for oxytocin was unknown and had to be established. To do so, 25 IU of oxytocin were administered intravenously to six cycling mares and blood samples were collected before and 2, 4, 8, and 15 min after administration. The half-life of oxytocin was determined to be 5.89 min, the clearance rate 11.67 L/min, mean residence time (MRT) 7.78 min. The effective plasma concentration was estimated to be 0.25 ng/mL. This was similar to the concentration achieved for the organ bath study where the concentration that produced 50% of the maximum effect (EC_{50}) was calculated at 0.45 ng/mL. To determine the intercyclic effect of oxytocin and carbetocin uterine myometrial samples were collected from slaughtered mares in estrus, diestrus, and anestrus. The samples were mounted in organ baths and exposed to four ascending, cumulative doses of oxytocin and carbetocin. Area under the curve and amplitude, maximum response (E_{max}), and concentration that produced 50% of the maximum effect were studied for each agonist and statistically evaluated. The effect of oxytocin on equine myometrial tissue was higher during diestrus, and surprisingly anestrus, than during estrus, whereas the effect of carbetocin was the same independent of the stage of estrous cycle. A significant difference was found for estrous and anestrous samples when oxytocin was used but not when carbetocin was used.

Keywords: Equine myometrium; Oxytocin; Carbetocin; Ex vivo; Organ bath

1. Introduction

Two major causes for economic losses in the equine breeding industry are the failure of a mare to conceive or a delay in a mare's reconception [1,2], both of which have shown to be caused mainly by a delayed uterine clearance in association with reduced uterine contractility [2]. Uterine contractions are vital in the transport of sperm through the uterus to the uterotubal junction, as well as for the clearance of sperm after breeding, or of accumulated intrauterine fluids [3]. A large percentage of mares susceptible to endometritis most likely have intrinsic contractile defects in myometrial contractility [4]. This results in accumulation of intrauterine fluid after breeding, predisposing the mare to persistent mating-induced endometritis (PMIE) [4]. In the mare, treatment of PMIE and stimulation of postpartum uter-

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ine involution [5] includes the use of oxytocin and natural or synthetic prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), which aid in promoting uterine clearance. Oxytocin stimulates uterine contractions, release of arachidonic acid, and prostaglandin formation, which in turn enhance uterine contractions by causing membrane depolarization and by increasing the number of gap junctions [6]. Clearance of uterine contents provides a uterine environment suitable for an embryo descending into the uterus, thus improving conception rates.

Both oxytocin and $PGF_{2\alpha}$ have disadvantages in their application; for example, oxytocin needs to be given in short intervals because of its short half-life (6.8 min), and $PGF_{2\alpha}$ in its natural form has unwanted side effects (sweating, tachypnea, increased gastrointestinal motility, signs of colic) [7,8]. A drug without side effects and a long half-life stimulating a prolonged tocolytic effect might improve uterine clearance. The use of carbetocin, a long acting analogue of oxytocin, has been investigated as a beneficial form of treatment on uterine involution in bovines [9], as well as in humans for the treatment of postpartum hemorrhage [10–12]. In adult, nonlactating anestrous mares carbetocin has been shown to have a half-life of approximately 17 min, which is 2.5-fold longer than that of oxytocin, and not associated with any side effects [7]. As such we believe that carbetocin may prove more beneficial in horses in inducing parturition, decreasing the time to conception postpartum, and aid in the treatment of PMIE [13].

For the current study we investigated if the concentration-dependent contractile responses of carbetocin in healthy estrous, diestrous, and anestrous equine myometrial smooth muscle were superior to that of oxytocin. The duration of effect was extrapolated from the pharmacokinetic time versus concentration profile for the mentioned oxytocin (self-generated) and carbetocin [7].

2. Materials and methods

2.1. Pharmacokinetic study

The pharmacokinetics of oxytocin was evaluated after a single intravenous dose in adult horses.

2.1.1. Animals

Six healthy, adult, nonlactating cycling Nooitgedacht mares of the Veterinary Faculty were used for the study. All animals were vaccinated and dewormed, and kept in their usual environment. The study was approved by the Animal Use and Care Committee of the University of Pretoria according the South African standard for the care of laboratory animals (SANS 10 386, Project Number V026/08).

2.1.2. Sample collection and preparation

An indwelling intravenous catheter was placed into the left jugular vein for blood sampling. The oxytocin treatment (25 IU) (Fentocin; Virbac, Centurion, South Africa) was administered into the opposite jugular vein by means of a disposable syringe and a 20-gauge needle. Blood samples were collected before administration and at 2, 4, 8, and 15 min after oxytocin administration, into chilled serum tubes containing aprotonin (500 kallikrein inhbitor unit (kIU)/mL of blood) (Aprotonin; Sigma-Aldrich (Pty), Ltd., Aston Manor, South Africa).

After collection, the blood samples were centrifuged at 1600 × *g* at 4 °C for 15 min and the supernatant of each sample was transferred to a labeled polycarbonate tube and immediately frozen at −80 °C. Hormone assay samples were extracted using a 200 mg C18 Sep-Pak (Waters Cooperation, Milford, MA, USA) column under the following conditions: equilibration of the column with 1 mL of acetonitrile, followed by 10 to 25 mL of 0.1% trifluoroacetic acid (TFA) in water before application of the sample. After sample application, the sample was washed with 10 to 20 mL of 0.1% TFA in water, followed by a final elution with 3 mL of acetonitrile 0.1% TFA in water (60:40). Samples were subsequently dried under a steady stream of nitrogen at 40 °C.

Oxytocin concentrations were quantified using the oxytocin Enzyme Immunoassay Kit (Assay Designs, Ann Arbor, MI, USA) according to the manufacturer's instructions. In short, 100 μL of sample were pipetted into sample wells with 50 μL of both the blue conjugate (alkaline phosphatase conjugated with oxytocin) and antibody. The plates were subsequently gently mixed and incubated at 4 °C for 18 to 24 h. After three washes with 400 μL of wash solution 5 μL of blue conjugate was added into to wells with 200 μL of p-nitrophenylphosphate in buffer (pNpp Substrate, Assay Designs, Ann Arbor, MI, USA) and allowed to incubate at room temperature for 1 h before the addition of 50 μL of Stop Solution. The 96-well plate was immediately read with an Eliza iEMS reader MF (Labsystems, Helsinki, Finland; wavelength range 340 to 850 nm, wavelength accuracy ± 2 nm) at an optical density of 405 nm with correction between 570 and 590 nm.

2.1.3. Pharmacokinetic analysis

Noncompartmental pharmacokinetic analysis was performed using Kinetica Version 5.0 (Thermo Fisher