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The distribution and influence of calcitonin gene-related peptide (CGRP) on the perfusion pressure in the isolated ovarian artery in the pig

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A moderate number of delicate CGRP-immunoreactive (CGRP-IR) varicose nerve terminals were found at the adventitia-media border of the sexually mature porcine ovarian artery by means of a routine single immunolabelling technique. Additionally, a pharmacological analysis was performed of the function fulfilled by α-CGRP and its C-terminal fragment (Tyr27)α-CGRP(27-37) in the porcine isolated ovarian artery, collected on days 8–13 of the oestrous cycle. It was shown that α-CGRP (10^{-8} M) caused a decrease in the perfusion pressure of the porcine isolated ovarian artery of 16% ($p < 0.05$), while (Tyr27)α-CGRP(27-37), added at the same concentration, reduced perfusion pressure by 13% ($p > 0.05$). Thus, we concluded that a-CGRP released from perivascular terminals may cause relaxation of the ovarian artery and, furthermore, that the potency of this action is dependent on the length of the chain of this peptide produced during the deactivation of the molecule by tissue proteases.

Key words: α-CGRP, (Tyr27)α-CGRP(27-37), ovarian artery, perfusion pressure, pig

INTRODUCTION

The adrenergic cholinergic and non-adrenergic non-cholinergic innervation of the porcine reproductive tract [4–7], as well as the role of acetylcholine, noradrenaline and neuropeptide Y in the regulation of blood flow in these organs has been well documented [1, 8]. Furthermore, studies performed over the last decade have shown that α-CGRP may also play an important role in the regulation of the vascular tone of the vessels supplying the reproductive tract [2]. As the presence of perivascular CGRP-IR nerve fibres has previously been described in the porcine female urogenital tract [5], the aim of the present study was 1) to determine the pattern of ovarian artery innervation by CGRP-containing nerve fibres and 2) to examine the influence of the exogenous α-CGRP and its C-terminal fragment (Tyr27)α-CGRP(27-37) on perfusion pressure in the porcine isolated ovarian artery.

MATERIAL AND METHODS

The genital organs were collected from mature (n = 18) sows in a local slaughterhouse. The days of the oestrous cycle were defined by macroscopic observation of the ovaries and the uterus. The preparations, including the ovarian artery and arteriovenous vascular network of the ovarian pedicle, together with the ovary, were isolated from the genital tract. The ovarian arteries were then cannulated and perfused with Krebs-Ringer's solution to remove

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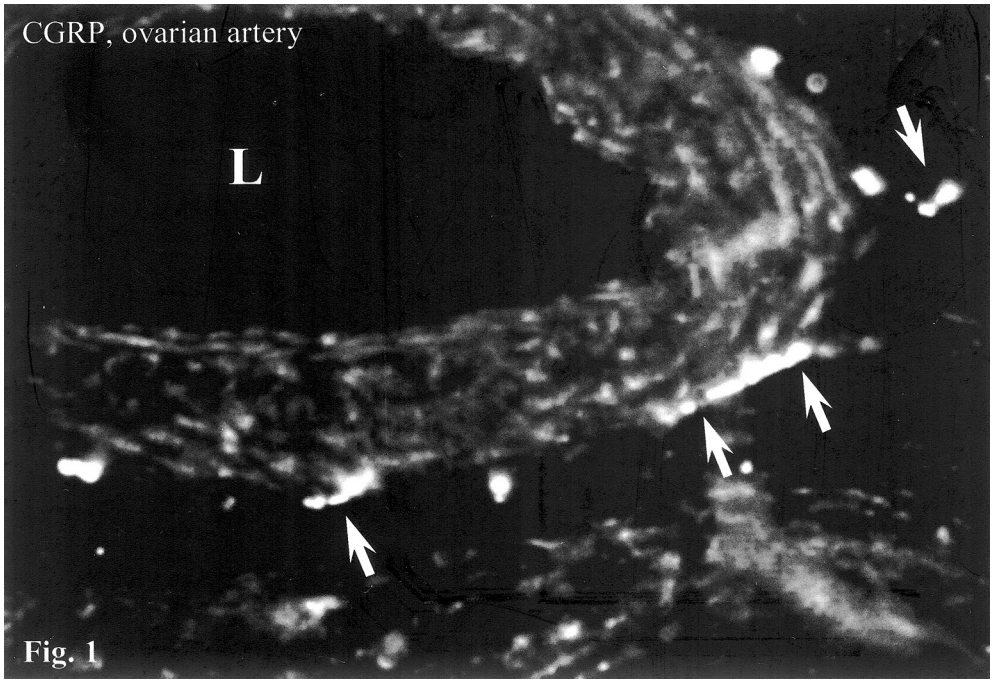

Figure 1. Distribution pattern of CGRP-IR nerve terminals (arrows) in the wall of the ovarian artery in a sexually mature gilt; L — lumen of the artery. × 200.

blood traces. Additionally, 1000 ml of the blood were collected from each animal to a heparinised bottle. The preparations were then placed into Krebs-Ringer's solution (composed of mM: NaCl — 120.3, KCl — 5.9, $CaCl_2$ — 2.5, $MgCl_2$ — 1.2, NaH_2PO_4 — 1.2, $NaHCO_3$ — 15.5, glucose 11.5; at 25°C, pH 7.4) and transported to the laboratory. Next the preparations were placed into the organ bath chamber, the cannulated ovarian artery was connected to a syringe pump (SEP21S, Medical Equipment Ascor, PL) and the specimen was perfused with its own oxygenated blood (5% CO_2 + 95% O_2, 37°C). Perfusion speed was calculated so as to allow the artificial perfusion pressure in the ovarian artery studied to reach 80–100 mm Hg. After stabilisation of the perfusion pressure the administration of the substances under investigation into the ovarian artery was started. Changes in the perfusion pressure were then measured using a physiological pressure transducer (type P23XL, Hugo Sachs Elektronik, GER) and registered using a multi-pen recorder type R-50 model 83 (Rikadenki, J). Different (10^{-9}-10^{-7} M) concentrations of α-CGRP or its C-terminal fragment (Tyr27) α--CGRP(27-37) were tested and the data obtained (before and after treatment) were analysed by t-tests (GraphPad Prism 3.0; San Diego, CA) and $p < 0.05$ was considered significantly different. The presence and distribution pattern of CGRP-IR varicose perivascular nerve fibres were disclosed by means of a routine single immunofluorescence technique.

RESULTS AND DISCUSSION

Immunofluorescence revealed a moderately dense mesh of CGRP-IR delicate varicose nerve terminals located at the adventitia-media border of the artery (Fig. 1). However, CGRP-IR material was not found in the endothelial cells. Preliminary results indicated that α-CGRP and (Tyr27)α-CGRP(27-37) at a concentration of 10^{-9} M (n = 2 specimens for each substance) did not cause changes in the perfusion pressure, while at a concentration of 10^{-7} M (n = 2) the perfusion pressure was reduced by 18.3% and 13.2% respectively. This was similar to the dose of 10^{-8} M (n = 5), which was used in the course of this study. Thus it was shown that this dose of α-CGRP reduced perfusion pressure in the porcine isolated ovarian artery by 16% ($p < 0.05$) and (Tyr27)α-CGRP(27-37) by 13% ($p > 0.05$) (Fig. 2). The results of the present study suggested that α-CGRP, most probably released from the perivascular nerve terminals, might also lead to a relaxation of the ovarian artery in the pig. The potency of this action is dependent on the length of the chain of this peptide, which is produced during deactiva-

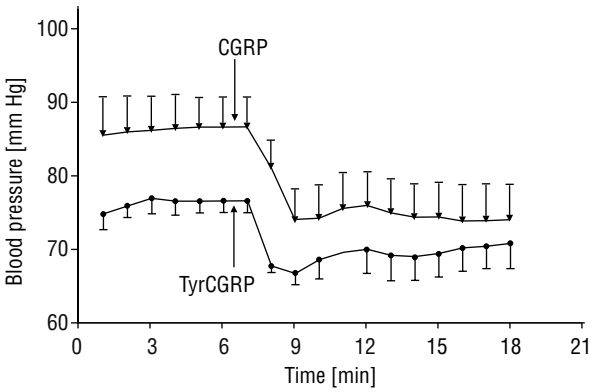

Figure. 2. Mean (± SEM) blood pressure in the porcine isolated ovarian artery on day 8–13 of the oestrous cycle after treatment with α-CGRP and (Tyr27)α-CGRP(27-37) in a concentration of 10^{-8} M (n = 10).

tion of the molecule by tissue proteases. A similar vasodilatatory action of α-CGRP was observed in the porcine isolated uterine artery [2] and the mesenteric vessels of the rat [3].

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