Tropical Agricultural Research Vol. 11 1999, 335-347

Local Secretion of Prostaglandin E2, Prostaglandin F2α, and Endothelin-1 in the Microdialysed Bovine Oviduct *In Vitro*: Effect of Luteinizing Hormone, Oxytocin, Endothelin-1, and Prostaglandins

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ABSTRACT. *Precise regulation of oviduct is essential to provide an environment for the successful sperm transport and capacitation, and oocyte transport and maturation, fertilization and early embryonic development. Thus, the effect of Luteinizing Hormone (LH), ovarian steroids and Oxytocin (OT), on the oviductal secretion of Prostaglandins (PG) and Endothelin (ET-1) was investigated using novel in vitro microdialysis system (MDS). In MDS the luminal epithelium of the oviduct collected during the post ovulatory stage was gently flushed by implanting a microdialysis capillary membrane. After 2 h pre-incubation, perfusates were collected every 4 h fractions until 16 h. At 4-8 h control (Ringers' solution only), progesterone (P4; 1 mg/ml), estradiol-17β (E2; 100 ng/ml), Luteinizing hormone (LH; 1 mg/ml), oxytocin (OT; 10^{-7}), ET-1 (10-7), PGE2 (10^{6}), PGF2α ($10^{}$) were infused. During follicular and periovulatory phases LH enhances the secretion of PGE2, PGF2α and ET-1. Furthermore, ET-1 stimulated the release of PGE2 and PGF2α but, none of the PG affected the ET-1 secretion. E2 showed a mild stimulation on PG and ET-1 secretion and P4 stimulated only the PGF2α secretion. However, any of these substances did not affect the basal release of PG or ET-1 during the luteal phase. Moreover, infusion of OT did not stimulates any of the measured substances during any phase of the estrous cycle. Thus, it can be suggested that local high levels of oviductal LH after the pre-ovulatory LH surge and the locally transferred E2 from the Graffian follicle may be important for oviductal production of PG and ET-1, which would activate oviductal contraction and the regulation of gamete/zygote transport through the oviduct.*

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INTRODUCTION

A precise regulation of oviduct is essential to provide an environment for the successful sperm transport and capacitation, oocyte transport and maturation, fertilization and early embryonic development. Different contractile patterns of the oviduct occur during the periovulatory period provide the foremost key role for these events to occur in the oviduct. It was observed that the low amplitude and frequency mortality patterns during the luteal phase were gradually increased and both amplitude and frequency reached a maximum during the estrus, but rapidly diminished over the next 3 days (Bennett *et al.*, 1988; Ruckebusch and Bayard, 1975). The cyclic variation of the countercurrent transfer of ovarian products to the oviduct is frequently claimed to be the source for these differences in the cyclic contraction patterns. The oviduct ipsilateral to the corpus luteum (CL) bearing ovary contained higher levels of progesterone (P4) and the developing follicle bearing ovary contained higher levels of estradiol 17β (E2), than that of the contralateral oviduct (Wijayagunawardane *et al.*, 1996; Wijayagunawardane *et al.*, 1998a). Moreover, oxytocin (OT) is also locally recirculated to the oviduct in higher concentration than that supplied in the peripheral circulation (Schramm *et al.*, 1986). On the other hand, it was reported that the oviduct of human (Han *et al.*, 1996; Lei *et al.*, 1993), swine (Derecka *et al.*, 1995) and cattle (Sun *et al.*, 1997) contain specific binding sites for LH, and a direct regulation by LH of the oviductal function has been postulated (Han, *et al.*, 1996; Lei *et al.*, 1993).

Our recent reports revealed that LH together with high levels of E2 and basal P4, induces the maximum stimulatory effect on oviductal PGE2, PGF2α and ET-1 production by the cow oviductal epithelial cell monolayers (Wijayagunawardane *et al.*, 1999a; Wijayagunawardane *et al.*, 1999b). Thus, this study is an attempt to evaluate the effect of LH, OT and ovarian steroid on the oviductal PG and ET-1 secretion using *in vitro* microdialysis system (MDS). For the above observation of oviductal secretion, the luminal epithelium of the oviduct was gently flushed through the implanted microdialysis capillary membrane. This system allows the maintenance of cell-to-cell integrity and thus cell-to-cell communication, and also enables the observation of real-time changes in the local release of different substances.

MATERIALS AND METHODS

Animals and sample collection

Reproductive tracts were collected from non-pregnant Holstein cows at a local slaughter house. The phase of the estrous cycle was determined according to previous reports (Wijayagunawardane *et al.*, 1996; Wijayagunawardane *et al.*, 1998a). The oviducts were separated from the utero-tubal junction and surrounding connective tissues were trimmed and then transported to the laboratory in Medium 199 (M 199; 25 mmol/l Hepes, 0.85 g/l NaHCO3, 60 mg/l penicillin, 100 mg/l streptomycin, 56 mg/l ascorbic acid and 2 mg/l amphotericin B; Sigma Chemical Co., St. Louis, MO) maintained at 38°C. The ampullary and isthmic regions of oviducts both ipsilateral and contralateral to the functioning CL or developing follicle and ovulation site were used in this study.

In vitro MDS of bovine oviduct

The *in vitro* MDS of the oviduct was adapted from the method described for the bovine CL (Miyamoto and Schams, 1991). Briefly, the lumen of each 10 cm long oviductal segment was implanted with a 7 cm long and 0.2 mm diameter dialysis capillary membrane (Fresenius SPS 600 Hollow fiber, Fresenius AG, St Wendel, Germany; cut off 1000 kDa) with each end glued to 5 cm long and 0.3 mm diameter silastic tubing. Both ends of the oviduct were then fixed to the silastic tubing by Histoacryl Blau (B. Braun Melsungen AG, Melsungen, Germany) and incubated in M199 with 0.5% BSA (Sigma Chemical Co., St. Louis, MO) in simple organ culture chambers (modified 50 ml falcon tubes; Becton Dickinson and Co,. NJ). Four oviducts were maintained in one organ culture chamber and the medium was continuously exchanged at a flow rate of 50 ml/h during the whole period of incubation. The chambers were maintained in a water bath at 38°C. Both ends of the silastic tubing were connected to Teflon tubing and Ringer's solution was continuously perfused (1.3 ml/h) from one end using a peristaltic pump, while the other end was connected to a fraction collector. After a 2 h pre-incubation, the perfusate was collected in 4 h fractions for 16 h. Control (Ringer's solution only) and LH (1 μg/ml: USDA-bLH-B-6; USDA Animal Hormone program), P4 (1 μg/ml: Sigma Chemical Co. St. Louis, MO), E2 (100 ng/ml: Sigma Chemical Co. St. Louis, MO), OT (10^{-7} M: Peptide Institute Inc., Osaka, Japan), ET-1 (10^{-7} M: Peptide Institute Inc., Osaka, Japan), PGE2 (10^{-6} M: Sigma Chemical Co. St. Louis, MO) and PGF2α (10^{-7} M: Sigma Chemical Co. St. Louis, MO) in Ringer's solution were infused at

4-8 h of incubation. The 4 h fractions of perfusate were stored in -20°C until extraction for PG and peptides.

Hormone extraction

PG and ET-1 extractions from MDS samples were performed as described earlier (Acosta *et al.*, 1998). As a result of the extractions, PG and ET-1 were concentrated 12 and 52 fold, respectively. Samples were dissolved in assay buffers for enzyme immunoassays (EIAs) for PG (40 mM PBS, 0.1% BSA, pH 7.2)and for peptide (42 mM NA2HPO4, 8 mM KH2PO4, 20 mM NaCl, 4.8 mM EDTA, 0.05% BSA, pH 7.5), respectively. The recovery rates, estimated by adding 3 different concentrations of PGE2 (1, 0.5, 0.1 ng/ml), PGF2α (1, 0.5, 0.1 ng/ml) and ET-1 (10, 5, 1 pg/ml) to the Ringers' solution were 78% for PGE2, 75% for PGF2α and 63% for ET-1.

Measurements of PG and ET-1

The second antibody EIAs for PGE2 (Wijayagunawardane *et al.*, 1998a) PGF2α (Miyamoto *et al.*, 1995), and ET-1 (Miyamoto *et al.*, 1997) were performed according to the methods developed in our laboratory as described previously. Within-assay and between-assay coefficients of variance (CVs) were 7.3% and 11.4% for PGE2, 8.2% and 11.8% for PGF2α and 9.6% and 13.2% for ET-1. The ED50's for the PGE2, PGF2α and ET-1 assays were 260 pg/ml, 355 pg/ml and 450 pg/ml, and the ranges of the standard curves for these assays were 0.03-14.2 ng/ml, 0.007-7.1 ng/ml and 10-10,000 pg/ml, respectively.

Statistical analysis

The basal release of PGE2, PGF2α and ET-1 in the MDS samples varied considerably among individual oviducts from different cows. Thus, the mean concentrations of each substance in the first 4 h of collection (fraction 1) were used to calculate the baseline for each individual (defined as 100%). Concentrations in subsequent fractions (fraction 2: 4-8 h, 3: 8-12 h and 4: 12-16 h) were expressed as a percentage of the individual baseline. The concentrations of PGE2, PGF2α and ET-1 in different fractions of different treatments and controls were analyzed using Fisher's PLSD test of analysis of variance (ANOVA) using Stat View™ (Abacusconcepts, 1992) computer software. Concentration among different fractions within a treatment were

analyzed using Student's T-test following F-test. Probabilities less than 0.05 ($P<0.05$) were considered significant.

RESULTS AND DISCUSSION

The basal release of PGE2, PGF2α and ET-1 (mean±SEM pg/ml Ringers' solution) in microdialysed oviducts were 534±210, 524±183 and 7.3±3.4 respectively for follicular phase, 629±154, 515±195 and 8.6±1.3 respectively for post ovulatory phase and 123±32, 185±30 and 3.4±0.4 respectively for luteal phase. The basal release of substances in the control groups were constant and stable during the experimental periods. The basal release of all substances measured was significantly higher during the follicular and postovulatory phases than during the luteal phase.

No significant differences were observed in the basal release or the stimulation by different substances between places within the oviduct (ampulla *vs* isthmas) or between sides (ipsilateral vs contralateral) of the oviducts from the same cyclic phase. Thus, the data from the same phase of the estrous cycle were combined to compare the secretory activity between different phases of the estrous cycle. In follicular and periovulatory phases, the LH enhanced the secretion of PGE2, PGF2α and ET-1. Furthermore, ET-1 stimulated the release of PGE2 and PGF2α but, none of the PG affected the ET-1 secretion. E2 showed a mild stimulation of PG and ET-1 secretion and, P4 stimulate only the PGF2α secretion (Figures 1-3). However, any of these substances did not affect the basal release of PG or ET-1 during the luteal phase (results not shown). Moreover infusion of OT did not stimulate any of the measured substances during any phase of the estrous cycle.

The phospholipase A2 activity and local oviductal production of PG were highest during the E2 dominant periovulatory period (Morishita *et al.*, 1993) and E2 was found to activate the PG synthetase in the cow uterus (Wlodawer *et al.*, 1976). The stimulatory effect of E2 on PG and ET-1 production also was observed in the present study. It was reported that the long term administration of P4 stimulates the uterine PGF2α production in ewes (Vincent and Inskeep, 1986). An increased PGF2α production with the P4 treatment was observed in the present study.

LH increases 5-lypoxygenase, cyclooxygenase (COX)-1 and COX-2 enzymes in the human oviduct (Han *et al.*, 1996; Lei *et al.*, 1993), and increases PG release in human umbilical cord (Rao *et al.*, 1993), cultured

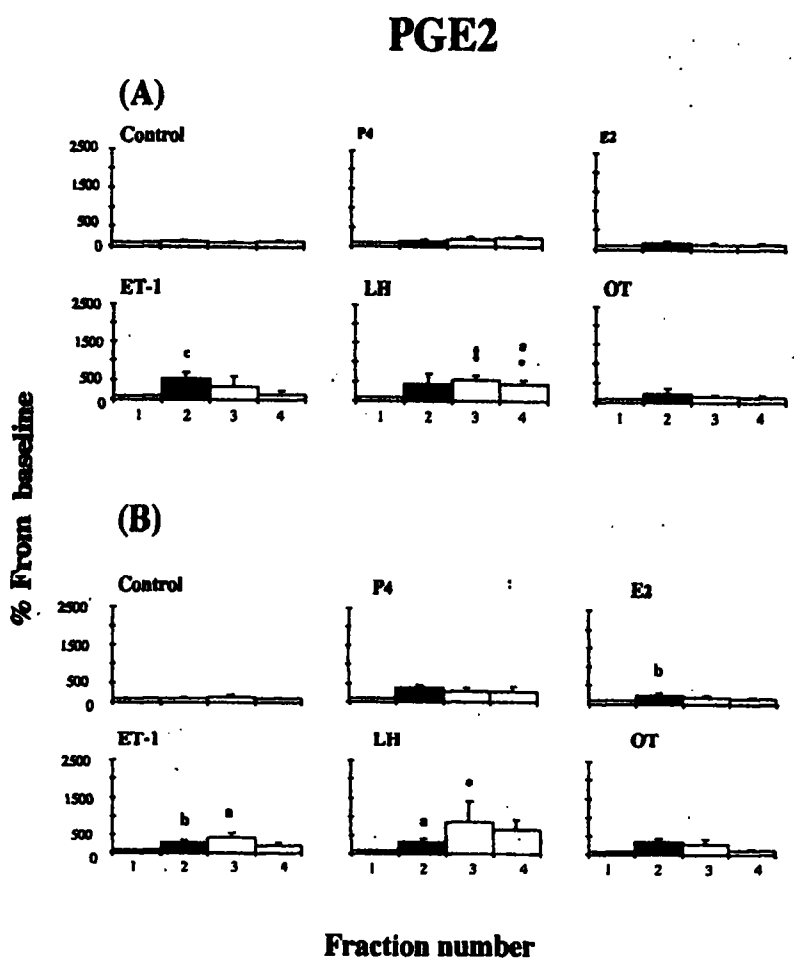

Figure 1. **Effect of progesterone (P4), estradiol 17β (E2), endothelin-1 (ET-1), luitinizing hormone (LH) and oxytocin (OT) on the prostaglandin E2 (PGE2) production by the oviducts collected during (A) follicular phase and (B) post ovulatory phase; n=12 for each phase.**

[Note: * Significantly different from controls; *P<0.05, **P<0.01; abc, Significantly different from values before treatments; a- P<0.05, b- P<0.01, c- P<0.001.

Basal release: Follicular phase = 534±210 pg/ml Ringers' solution ± SEM
Post ovulatory phase = 629±154 pg/ml Ringers' solution ± SEM]

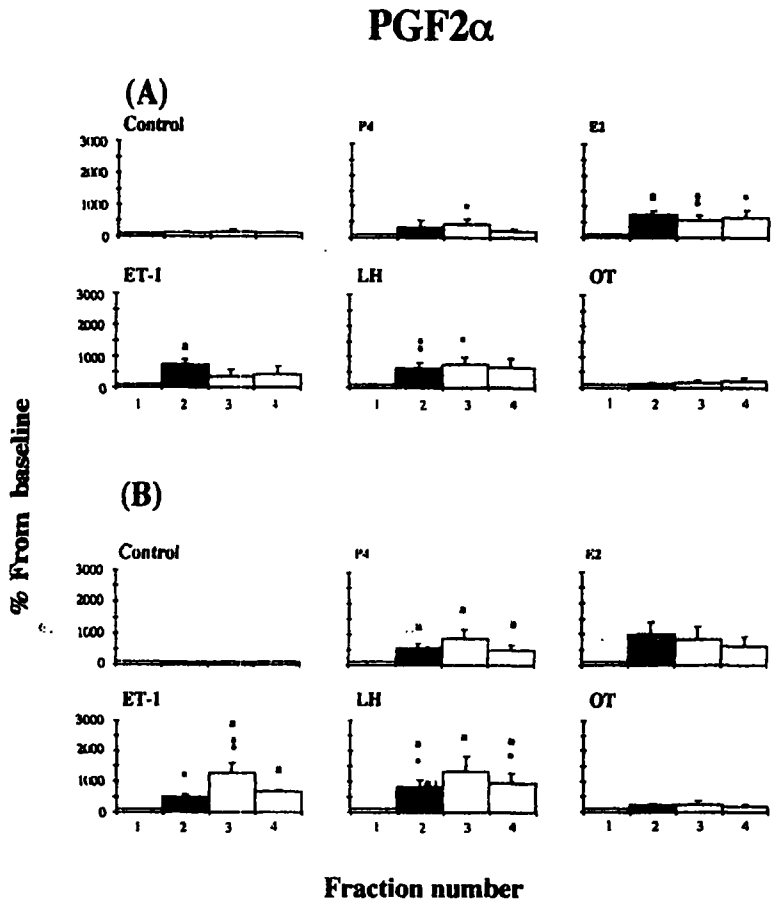

Figure 2. **Effect of progesterone (P4), estradiol 17β (E2), endothelin-1 (ET-1), luitinizing hormone (LH) and oxytocin (OT) on the prostaglandin F2α (PGF2α) production by the oviducts collected during (A) follicular phase and (B) post ovulatory phase; n=12 for each phase.**

[Note: * Significantly different from controls; *P<0.05, **P<0.01. a, Significantly different from values before treatments; P<0.05.

Basal release: Follicular phase = 524±183 pg/ml Ringers' solution ± SEM
Post ovulatory phase = 515±195 pg/ml Ringers' solution ± SEM]

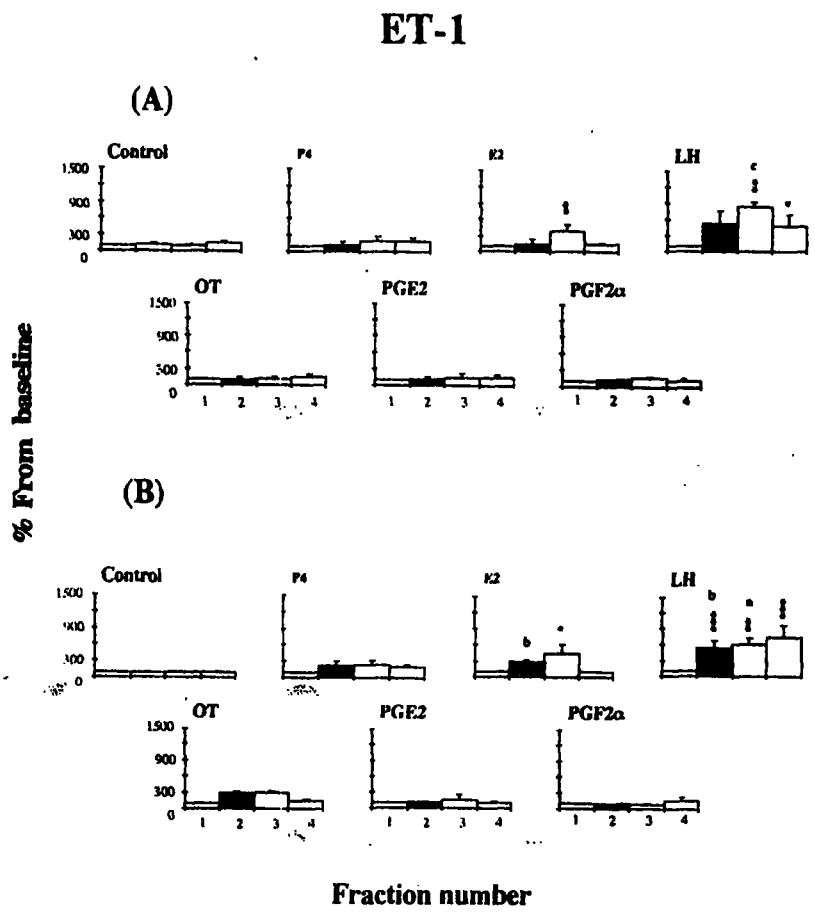

Figure 3. **Effect of progesterone (P4), estradiol 17β (E2), luitinizing hormone (LH), oxytocin (OT), prostaglandin F2α (PGF2α) and prostaglandin E2 (PGE2) on the endothelin-1 (ET-1) production by the oviducts collected during (A) follicular phase and (B) post ovulatory phase; n=12 for each phase.**
[Note: * Significantly different from controls; *P<0.05, **P<0.01; ***P<0.001. abc, Significantly different from values before treatments; a- P<0.05, b- P<0.01, c- P<0.001.
Basal release: Follicular phase = 7.3±3.4 pg/ml Ringers' solution ± SEM
Post ovulatory phase = 8.6±1.3 pg/ml Ringers' solution ± SEM]

endothelial cells (Shemesh *et al.*, 1997) and human fetal membranes (Toth *et al.*, 1996). Moreover, LH activates protein kinase (PK)-A and PK-C in porcine myometrial smooth muscle cells (Kisielewska *et al.*, 1996) and results in increased ET-1 mRNA levels in cultured bovine endothelial cells (Emori *et al.*, 1991). Therefore, the stimulatory effect of LH on the oviductal production of PGE2, PGF2α and ET-1 may be due to the activation of PK and COX.

It was reported that ET-1 activates multiple biochemical pathways including phospholipase (PL)-C, PL-D and the arachidonate cascade within the target cells, and that PL-D plays an important role in the ET-1 stimulation of 6-keto-PGF1α release (Liu *et al.*, 1992). ET-1 stimulates calcium channels, phospholipase (PL)-C and PL-A2 in smooth muscle cells through activation of the ET receptors (Masaki *et al.*, 1990). In the present study, ET-1 significantly increased PG secretion by the oviduct, although neither PGE2 nor PGF2α affected ET-1 production. The above observations suggest that the ET-1 produced by the oviduct may serve as a paracrine modulator of oviductal function, and may play a major role in the control of local oviductal contraction. It appears that ET-1 is a local mediator of LH that stimulates PG secretion during the periovulatory period, in addition to directly acting on the contraction of oviductal smooth muscles.

The local oviductal tissue levels of OT exceeded its plasma concentrations (Lundin *et al.*, 1989) and, oviductal musculature was most sensitive to physiological concentrations of OT at the estrus (Gilbert *et al.*, 1992). On the other hand, it was also reported that the stimulatory effect of OT on oviductal contraction is minimal at ovulation and thus it seems unlikely that OT is involved in control of ovum transport (Harper, 1988). We recently reported that the local distribution of OT in the bovine oviduct is low and that it did not vary by the stage of the estrus cycle (Wijayagunawardane *et al.*, 1998a). Furthermore, OT did not affect PGF2α or PGE2 output from the guinea-pig endometrium maintained in tissue culture (Riley and Poyser, 1989). In the present study, similar results that OT lacked an effect on oviductal PGE2, PGF2α and ET-1 production were observed.

A high concentration of P4 during the luteal phase makes the tissue less responsive to ovarian E2 (Henricks and Harris, 1978) and blocks the effect of E2 on protein secretion (Buhi *et al.*, 1992) and oviductal epithelial cell differentiation (Kamwanja and Hansen, 1993). P4 reduces PG production by lowering PL-A2 activity (Morishita *et al.*, 1993). The human fallopian tubes in the secretory phase contain more hCG/LH-R than in the proliferative phase (Lei *et al.*, 1993). E2 promoted the synthesis of LH/hCG receptors in

pig myometrium (Flowers *et al.*, 1991). The ovarian steroid hormones may play a role in the control of expression of ET-R in the human endometrium (Collett *et al.*, 1996), and oviductal ET-1 levels are highest during the periovulatory period (Wijayagunawardane *et al.*, 1998a). Moreover, LH stimulated oviductal contraction was only observed during the periovulatory period and LH exert no effect on oviduct contraction during the luteal phase (Wijayagunawardane *et al.*, 1998b). Thus, the lack of stimulatory effects of substances infused on the PG and ET-1 release in the luteal phase oviducts may be due to the low levels of cellular differentiation and binding sites during this period.

CONCLUSIONS

The results suggest that the LH and E2 stimulate PG and ET-1 release from the bovine oviduct during the peri ovulatory period. Moreover, ET-1 is an important stimulator of PG secretion. Thus ET-1 may act as a local intermediator of LH modulation of oviductal PG secretion. The overall results suggest that local high levels of oviductal LH co-incidence with the pre-ovulatory LH surge and the locally transferred E2 from the Graffian follicle may be important for oviductal production of PG and ET-1, thus, the active oviductal contraction and the regulation of gamete/zygote transport through the oviduct.

ACKNOWLEDGMENTS

The authors wish to thank Dr. D. Schams, Technical University of Munich, Germany, for ET-1 antiserum, Dr. S. Ito, Kansai University of Medicine, Japan, for PG antiserum, Dr. D.J. Bolt, USDA Animal Hormone program, for bovine LH, and Fresenius AG, St Wendal, Germany for the microdialysis capillary membranes.

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