

Parke-Davis Report (1992) - MECLOFENAMATE

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Experiment: The effect of *meclofenamate* on the isolated rat uterus perfused with *Res-Del* RS-I mammalian solution in a *Res-Del* 589 Organ Perfusion Bath.

INTRODUCTION

The versatile design of the *Res-Del* Perfusion Bath System (see Figs. 1,4 & 5) allows for a limitless variety of tissue/organ preparations from different mammalian species to be utilised in physiological and pharmacological experimentation (Rees, 1989 a,b) far in excess of that currently afforded by older, *vertically* designed organ chamber apparatus.

The *Res-Del* Perfusion Bath with its *Sylgard* resin-base floor allows for different types of visceral and skeletal nerve-muscle preparations to be securely positioned horizontally within the central compartment (see Figs. 4,5 & 14) to measure both mechanical and electrophysiological events under varying physiological and pharmacological conditions.

The advantageous horizontal alignment of tissue/organ preparations within the *Res-Del* perfusion bath allows for stereozoom microscopes to be used to further aid the operator **for ongoing**, visual inspection, dissection, biopsy acquisition and mechanical and electrical manipulative procedures to be performed on isolated preparations without unduly disturbing their functional activity.

The superior flow, aeration and temperature control features incorporated into the *Res-Del* perfusion/perifusion bath has been proven in teaching and research experimentation to sustain the functional viability of isolated tissues stored **non-**perifused at 8-12 °C for several days (**1-11**) and equally, tissues and organs per fused/peri fused at *in situ* temperatures for up to 3 days using *Res-Del* RS-I mammalian solution.

In comparison to non-perifused, *vertical* organ chamber systems which use a myriad of taps, tubes and coils to control the environment of *quasi-physiological* salines, formulated over the last 80 years, *Res-Del* technology has been demonstrated to significantly reduce (> 60%) the number of animals sacrificed in both teaching and research simply because the same technology can accommodate and physiologically maintain over extended *in vitro* time periods (eg. **1-14 days**), the heart, lung, liver, kidney and visceral/skeletal tissue preparations from the same animal, eg. mouse, rat, guinea pig.

EXPERIMENTAL METHODOLOGY

Protocol

Peri fusion of isolated uterus preparations from diestrus/oestrogen-sensitized rats previously stored @ 8 °C for 1 - 6 **days** in *Res-Del* RS-I mammalian solution and examined in a *Res-Del* 589 perfusion bath at 35.0 ± 0.4°C.

Preparations

All uterine preparations examined in the *Res-Del* 589 perfusion bath were aligned horizontally within the central compartment of the bath (Fig.4) and totally submerged during perfusion of *Res-Del* RS-I mammalian solution which was continuously aerated with *carbogen* (95% CO₂ / 5% O₂).

Contractility

Grass FT 03 isometric force-displacement transducers were aligned horizontally (Fig.14) to measure changes in length tension of the uterine muscle preparations. Each 1.0 cm long uterine preparation was secured to the *Sylgard* resin-base floor of the central compartment with fine steel pins (Fig.5) and then attached to the transducer rod at T... 1.0 g.

Perfusate

Res-Del RS-I mammalian solution: **10x** concentrate; batch No.9103; expiry 08/89

(Immuno-chemical Products Ltd, NZ)

Pharmacology

(a) *Techniques*

All pharmacological agents were administered at their final concentrations using *Res-Del* RS-I mammalian solution as the solvent medium and either,

- (i) perfused/perifused at 1 - 40 ml/min at the experimental temperature, or,
- (ii) topically applied **at** the experimental temperature via a micropipette positioned within 5 **mm** of the 'target^f' area of the preparation during perfusion of the bath at 4.0 ml/min.

(b) *Drugs*

Meclofenamate Sodium Monohydrate: Lot A, PD 45330-15K;

(Parke-Davis, USA)

Oxytocin: (Lot 44F-0332; Sigma Chemical Co., USA)

Prostaglandin PGF_{Zot} (Sigma Chemical Co., USA)

Indomethacin; (Sigma Chemical Co., USA)

EXPERIMENTAL RESULTS

Experiments E.1/E.2:

Preliminary experiments demonstrate the retention of both receptor sensitivity to epinephrine, norepinephrine and acetylcholine and inherent contractility of uterine preparations from the diestrus rat after storage @ 8 °C for 1-6 days and/or perfusion @ 15-35 ± 0.4 °C using *Res-Del* perfusion technology.

Experiment E.3:

This experiment was conducted by 3rd-year neuropharmacology students utilising a *Res-Del* 589 perfusion bath assembly (see Figs. 1, 4 & 5) whereby simultaneous examination of *control*(C) and *experimental* (E) rat uterus preparations was conducted under the same controlled conditions of perfusion flow and temperature.

In this experimental procedure indomethacin was used to block the prostaglandin-mediated contractions in the oestrogen-sensitised rat uterus.

The evoked contractures to topically applied oxytocin and $\text{PGF}_{2\text{ot}}$, shown in both fresh biopsies of the rat uterus and after they were perfused @ 25.0 ± 0.4 °C for 16 **hrs** with 5×10^{-7} M indomethacin, suggests that oxytocin acts both directly and indirectly (via PCs) to stimulate myometrium contractility in the rat uterus.

Experiment E.4:

The dose responsiveness of the diestrus rat uterus to topically applied oxytocin is shown under the controlled conditions of perfusion flow and temperature afforded by the *Res-Del* perfusion bath.

Various doses of meclofenamate were tried to ascertain an affective concentration that would, during perfusion of this drug, result in simultaneous inhibition of PG-synthesis and blockade of PG-receptors as reported by Bernal et al.(1991). As demonstrated by these authors, meclofenamate at 10^{-6} M resulted in a fast (< 1 min) cessation of spontaneous contractility which was sustained over 15 min during perfusion of the drug.

Interestingly, there appeared to develop over this experimental period a 100-fold decrease in the oxytocin receptor responsiveness. Whether this was due to a *down-regulation* of the PG-receptors as suggested by Bernal et al.(1991) for concentrations of meclofenamate > 10^{-5} M would need to be checked by topical application of PGF_2^* and PGE_2 .

The final phase of this experiment demonstrated that the observed decreased responsiveness to oxytocin was completely reversed upon re-perfusion over a 30 min period with *Res-Del* RS-I mammalian solution.

Experiment E.5:

In another set of experiments the effect of perfusing $2.5-5 \times 10^{-6}$ M meclofenamate was investigated on uterine preparations stored for 2 and 6 days *in vitro* @ 8 °C in *Res-Del* RS-I mammalian solution.

In brief, the latter results indicated that the effectiveness of meclofenamate in blocking oxytocin-induced contractures is still evident in long-term stored preparations and that even during 50 min perfusion with 5×10^{-6} M meclofenamate the response to topically applied oxytocin was retained.

COMMENT: The rat uterus experiments (E.1-5) indicate that even after 1 - 11 days storage at 8 °C in *Res-Del* RS-I solution, inherent rhythmicity and pharmacological responsiveness in these uterine biopsies persisted for a further 2 days during perfusion at 15 - 35 °C.

Retention of *receptor-specific* action was evident in uterus biopsies from diestrus/oestrogen-sensitized rats, whereby, it can be seen that while the spontaneous contractility that persists after storage in *Res-Del* RS-I mammalian solution is effectively blocked by meclofenamate, an inhibitor of both prostaglandin synthesis and PGF_2 receptors, the sensitivity of the *oxytocin-receptors* to micromolar concentrations of topically applied oxytocin was still evident.

Rees, D. (1989a). Consideration of the inorganic and organic composition of mammalian perfusion solutions. In: Doring HJ & Dehnert H, eds. *Isolated Perfused Organ Preparations*. March: Biomesstechnik-Verlag March GmbH, 5: pp 85-94.

Rees, D: (1989b). *Isolated Perfused Kidney*. In: Doring HJ & Dehnert H, eds. *Isolated Perfused Organ Preparations*. March: Biomesstechnik-Verlag March GmbH, 5: pp 123-132.

Bernal, A.L, Buckley, S., Rees, CM.P. & Marshall, J.M. (1991). Meclofenamate inhibits prostaglandin E binding and adenylyl cyclase activation in human myometrium. *Journal of Endocrinology* 129, 439-445.

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E. 1 Functional viability of isolated rat (DIESTRUS) uterus preparations following long-term storage at 8°C in *Res-DeI*® *RS-I* mammalian solution

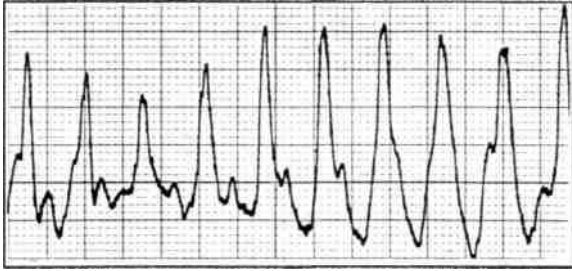
Date: 26-06-86

Animal species: *Rattus norvegicus*

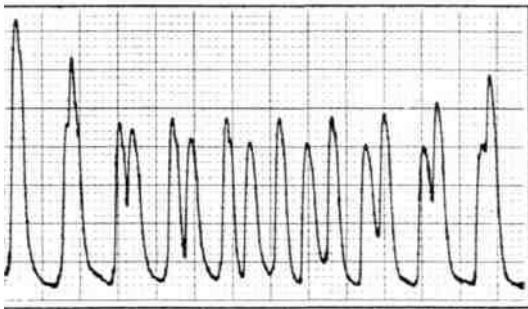
Uterine preparation stored @ 8.0 °C for 7 days in *RS-I* mammalian solution

CHART DIRECTION - LEFT to RIGHT

cal. - time base 1.5 cm/min
amplitude 1.0 g/cm

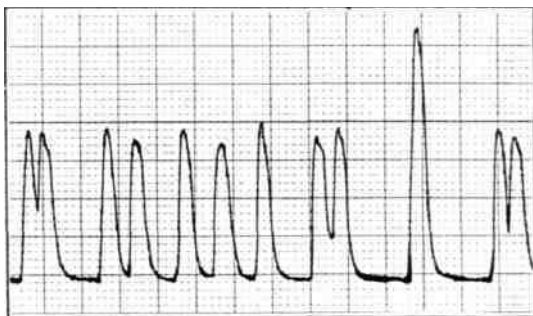


after 15 mins @ 35.2 + 0.4 °C pH 7.44

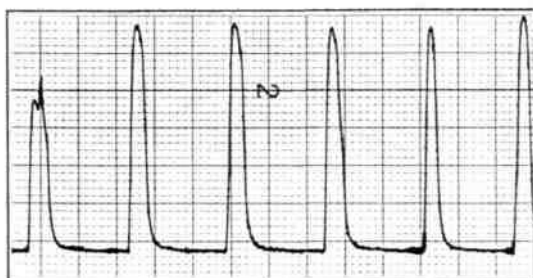


after 45 mins @ 38.4 ± 0.4 °C
pH 7.49

after 25 mins @ 35.4 ± 0.4 °C
pH 7.45



Perfused with *RS-I* @ 15.2 + 0.4 °C for 22 hours



after 45 mins @ 35.2 ± 0.4 °C
pH 7.42

K. 2 Functional viability of isolated rat (DIESTRUS) uterus preparations following **long-term** storage at 8°C in *Res-DeI® RS-I* mammalian solution

Date: **30-06-86**

Animal species: *Rattus norvegicus*

Preparation: ovary + horn

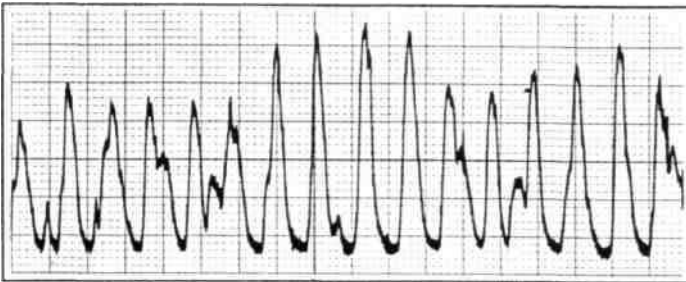
Exp. cond: Uterine preparation stored @ 8.0 °C for 11 days in *RS-I* mammalian solution and examined @ 35.0 + 0.4 °C

Days in vitro = 11

CHART DIRECTION - LEFT to RIGHT

cal. - time base: 1.0 cm/min
amplitude: 0.5 g/cm

after 90 mins perfusion

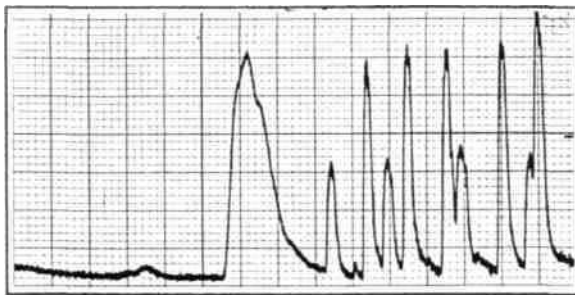


after 180 mins perfusion



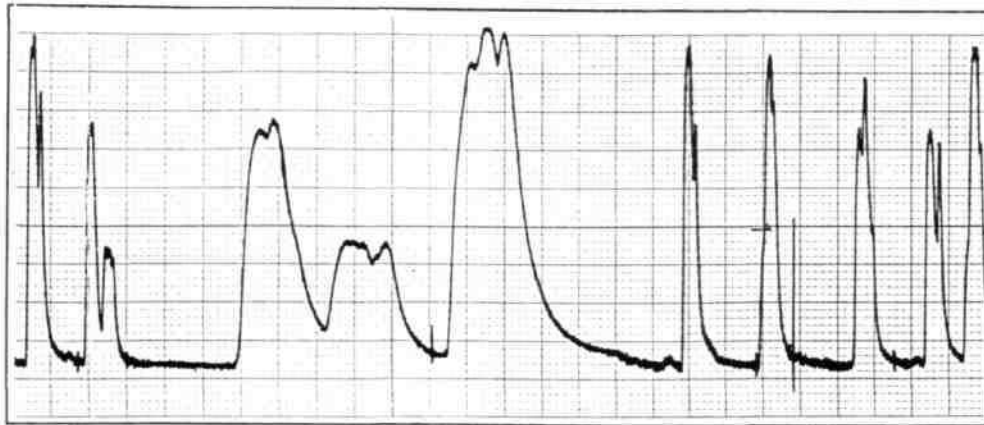
10⁻⁵ M adrenaline - topically applied during perfusion of *RS-I* @ 1.0 ml/min

after 210 mins perfusion



10⁻⁵ M noradrenaline - topically applied during perfusion of *RS-I* @ 1.0 ml/min

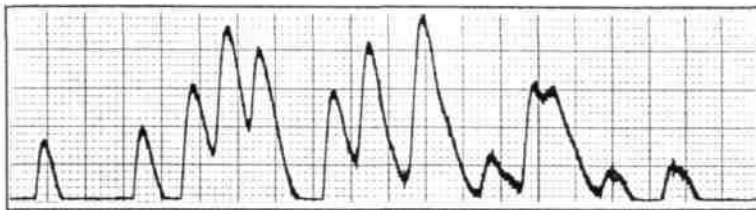
after 240 mins perfusion



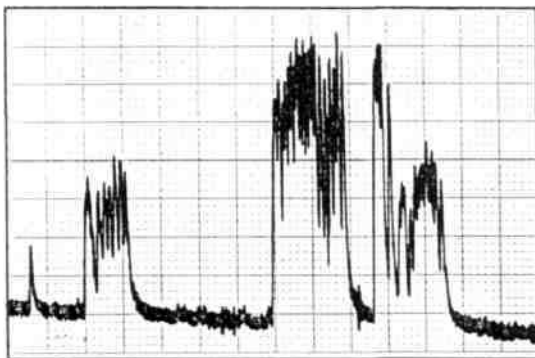
■
 10^{-5} M acetylcholine

Preparation perfused (5) 1.0 ml/min with *RS-I* (g) 15.2 ± 0.4 °C for 22 hours

Date: 01-07-86 Days *in vitro* = 12



@ 20 ± 0.4 °C



@ 30 ± 0.4 °C



@ 37.0 ± 0.4 °C

1.0 g

Preparation perfused @ 1.0 ml/min with RS-I @ 15.2 ± 0.4 °C for 22 hours

Date: 02-07-86 Days in vitro = 13

perfused @ 1.0 ml/min with RS-I (5) 35.2 + 0.4 °C



after 15 mins perfusion

Preparation perfused @ 4.0 ml/min with 5 x 10⁶ M indomethacin @ 35 + 0.4 °C



■ ON
5 x 10⁻⁶ M indomethacin



After 60 mins perfused with
5 x 10⁻⁶ M indomethacin

120sec

E. 3 The effect of oxytocin, $PGF_{2\alpha}$, with/without indomethacin on the isolated rat (OESTROGEN-SENSITISED) uterus perfused with *Res-De/® RS-I* mammalian solution

Date: 11-09-90

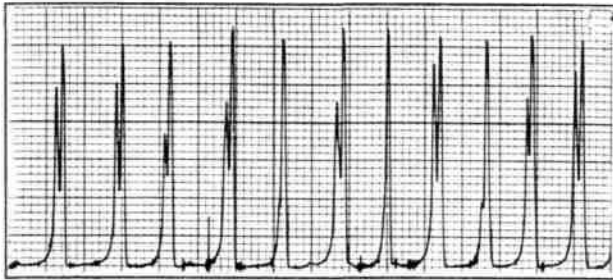
Animal species: *Rattus norvegicus*

Exp. cond: LEFT *Res-De/®* perfusion bath = **Control (C)**

RIGHT *Res-De/®* perfusion bath = **EXPERIMENTAL (E)**

CHART DIRECTION - RIGHT to LEFT

cat.- time base: 0.5cm/min
amplitude: 1.0 g/cm



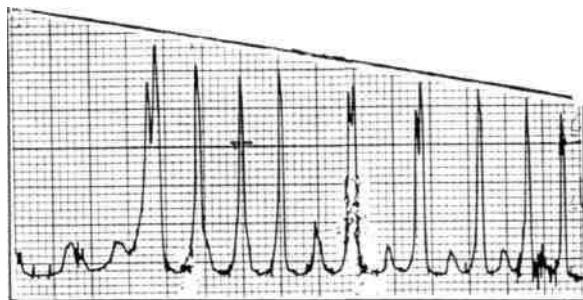
(E)



(C)

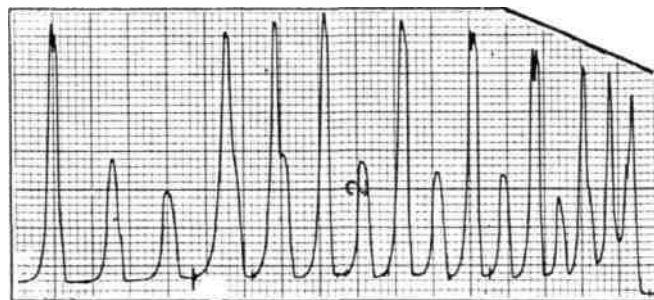
2.0 g

after 25 mins perfusion (1.0ml/min) in *RS-I* mammalian solution @ 35.0 ± 0.4 °C



(E)

2.5 x 10⁻⁶ M oxytocin (topically applied)



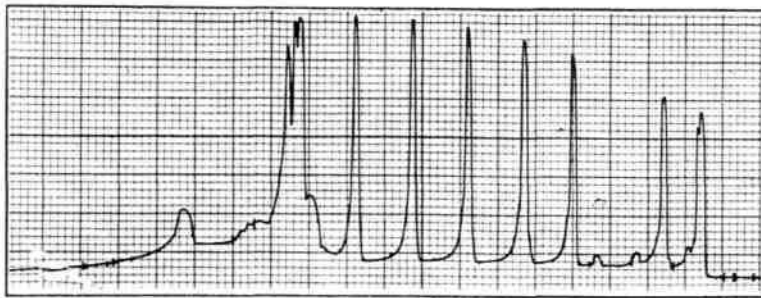
(C)

2.0 g

2.5 x 10⁻⁶ M oxytocin (topically applied)

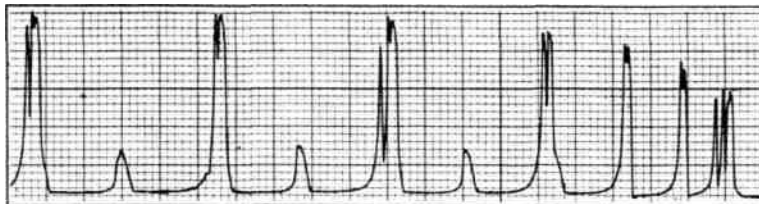
after 15 mins re-equilibration in *RS-I* mammalian solution @ 35.0 ± 0.4 °C

CHART DIRECTION - RIGHT to LEFT



(E)

10⁻⁸ g/ml PGF_{2α} (topically applied)



(C)

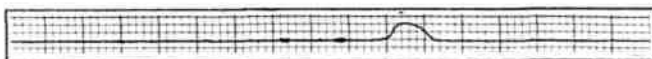
2.0 g

10⁻⁸ g/ml PGF_{2α} (topically applied)

(C) preparation perfused overnight in *RS-I* mammalian solution @ 25.0 ± 0.4 °C
(E) preparation perfused overnight in *RS-I* mammalian solution + 5×10^{-7} M indomethacin @ 25.0 ± 0.4 °C

Date: 12-09-89 Days
in vitro = 1

after 2 hrs perfusion with *RS-I* + 5×10^{-7} M indomethacin (P > 24.6 + 0.4 °C)



(E)



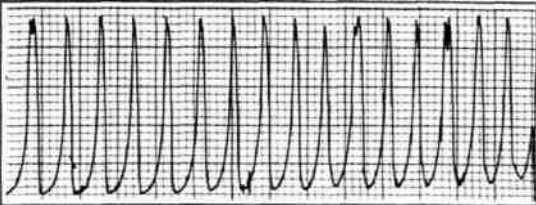
(C)

2.0 g

increase temperature of (C) and (E) perfusates to $35.0 \pm 0.4 \text{ }^\circ\text{C}$

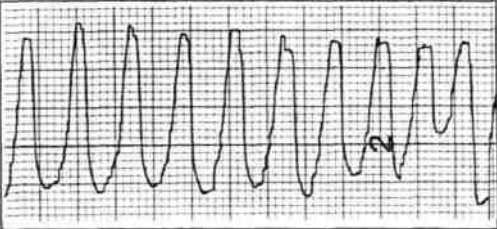
CHART DIRECTION - RIGHT to LEFT

Drugs applied topically during perfusion of (C) and (E) baths @ 1.0 ml/min



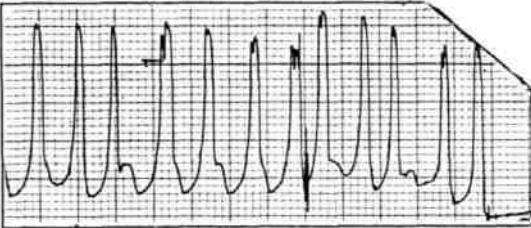
(E) spontaneous contractility blocked for 15 hrs in *RS-I* + 5×10^{-7} M indomethacin

■
 2.5×10^{-6} M oxytocin (topically applied)



(C) *RS-I*

■
 2.5×10^{-6} M oxytocin (topically applied)



(E) spontaneous contractility blocked for 16 hrs in *RS-I* + 5×10^{-7} M indomethacin

■
 10^{-8} g/ml $\text{PGF}_{2\alpha}$ (topically applied)



(C) *RS-I*

2.0 g

■
 10^{-8} g/ml $\text{PGF}_{2\alpha}$ (topically applied)

- 4 The effect of oxytocin with/without Meclofenamate on the isolated rat (*DIESTRUS*) uterus perfused with *Res-De/® RS-I* mammalian solution @ 35.0 ± 0.4 °C

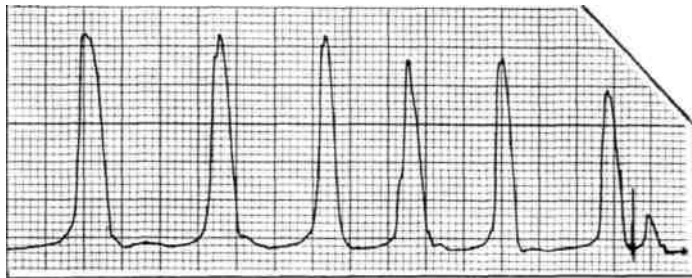
Date: 08-10-91

Animal species: *Rattus norvegicus*

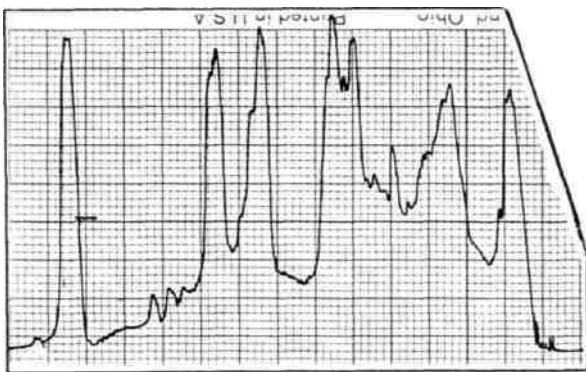
Exp. cond: Uterine preparation examined @ 35.0 ± 0.4 °C in *RS-I* mammalian solution

CHART DIRECTION - RIGHT TO LEFT

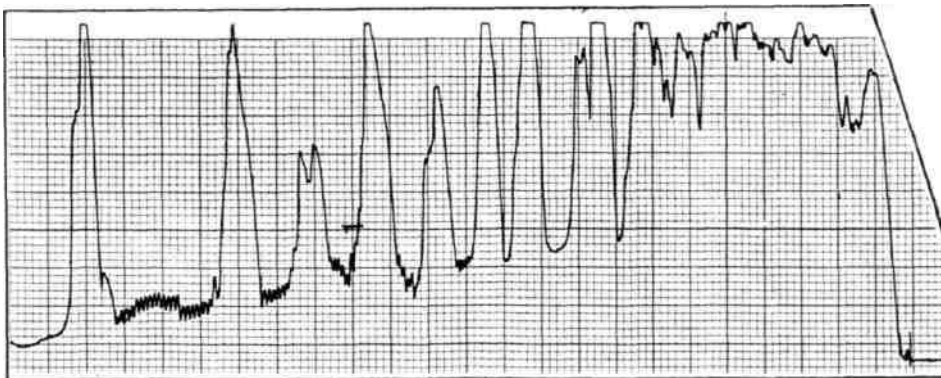
cal.- time base: 1.5 cm/min
amplitude: 1.0 g/cm



■
 2.5×10^{-8} M oxytocin

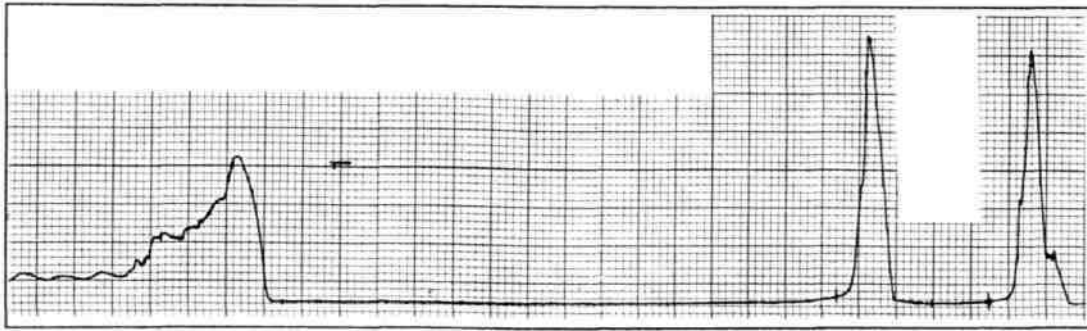


■
 2.5×10^{-7} M oxytocin

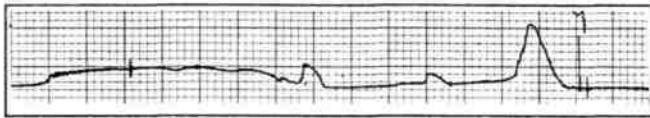


■
 2.5×10^{-6} M oxytocin

CHART DIRECTION - RIGHT TO LEFT



■ 2.5×10^{-8} M oxytocin ■
spontaneous contractions
BLOCKED after 1.0 min 10^{-4} M meclofenamate



after 15 mins. perfusion with
 10^{-4} M meclofenamate

■ 2.5×10^{-6} M oxytocin

10^{-4} M meclofenamate washed off during perfusion with *RS-I* @ 4.0 ml/min



after 10 mins

■ 2.5×10^{-6} M oxytocin



after 30 mins

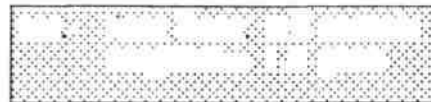
■ 2.5×10^{-6} M oxytocin

E. 5 The effect of oxytocin with/without Meclofenamate on the isolated rat (DIESTRUS) uterus perfused with *Res-DeI® RS-I* mammalian solution

Date: 09-08-91

Animal species: *Rattus norvegicus*

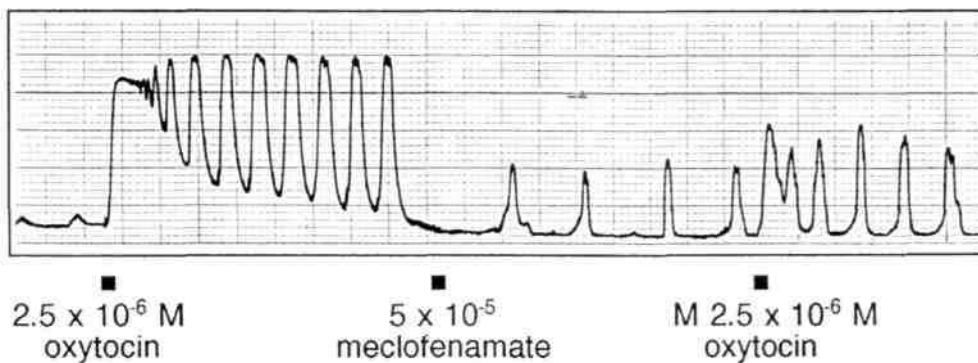
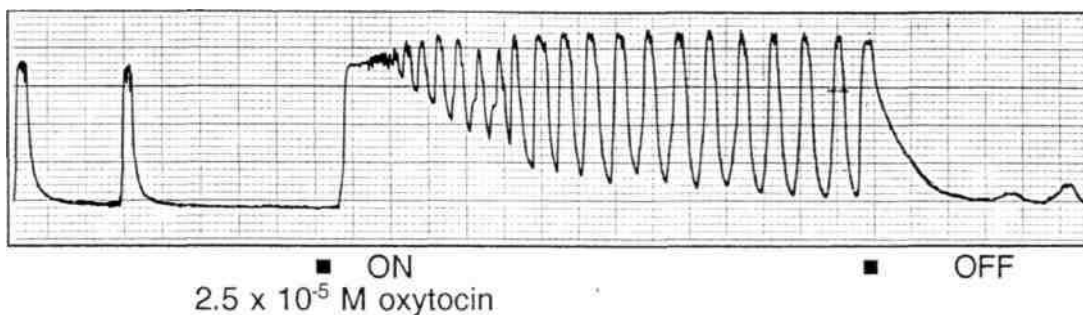
CHART DIRECTION - LEFT to RIGHT



time base 10^0 cm/min
amplitude: 1,0 g/cm

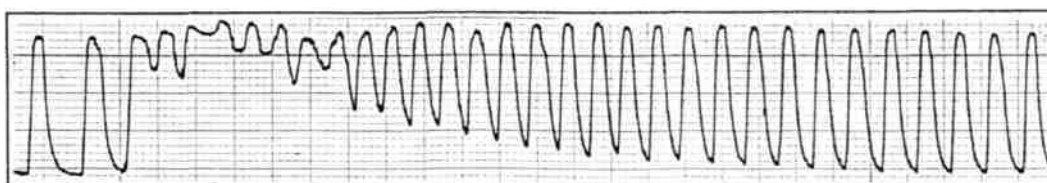
Left uterine preparation stored @ 8.0 °C for 2 days in *RS-I* mammalian solution

(5) 35.0 ± 0.4 °C



Right uterine preparation stored @ 8.0 °C for 6 days in *RS-I* mammalian solution

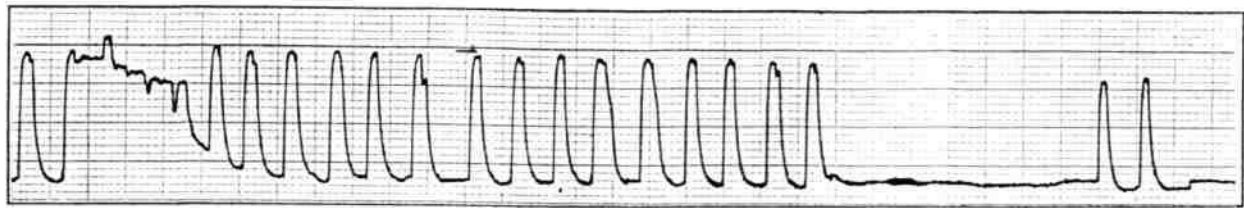
(g) 35.0 ± 0.4 °C



2.5 x 10⁻⁶ M oxytocin - topically applied during perfusion @ 4.0 ml/min

CHART DIRECTION - LEFT to RIGHT

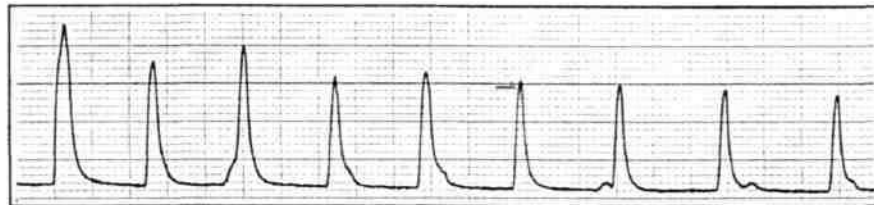
after 25 mins perfusion with *RS-1* (5) 4.0 ml/min @ 35.0 + 0.4 °C



■
 2.5×10^{-7} M
oxytocin

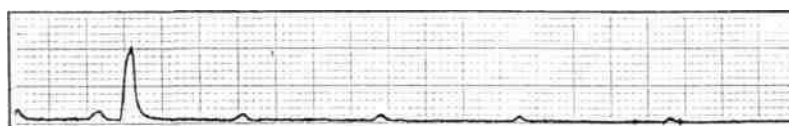
■
STOP perfusion and
ADD 5×10^{-5} M meclofenamate

after 20 min perfusion with 10^5 M meclofenamate



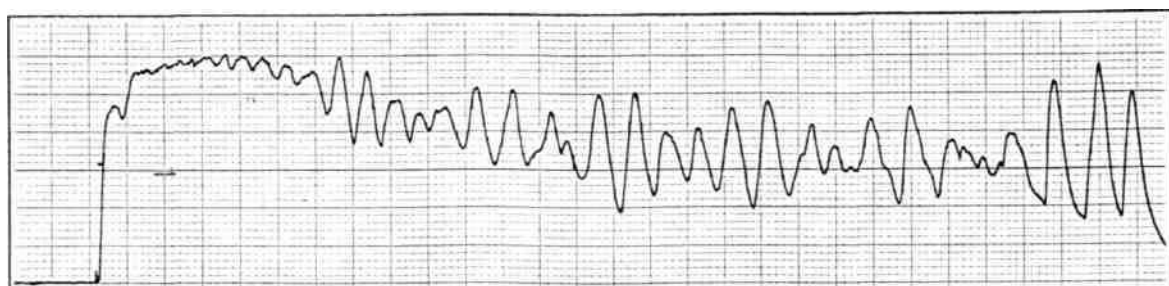
■
 $> 2 \times 10^{-5}$ M meclofenamate

after 20 min perfusion with 2×10^5 M meclofenamate



■
 $> 5 \times 10^5$ M meclofenamate

after 10 min perfusion with 5×10^5 M meclofenamate



■
 2.5×10^{-6} M oxytocin - topically applied to meclofenamate-blocked uterus