Effects of arsenite and oxamate on the in vitro functional activity of estrogen-dominated rat uterine horns[1]

A.L. Gimeno[2], A. Goldraij[3] and M.F. Gimeno[2]

Centro de Estudios Farmacológicos y de Principios Naturales (CEFAPRIN), Consejo Nacional de Investigaciones Científicas y Técnicas, Obligado 2490, Buenos Aires 1428 (Argentina), and Departamento de Fisiología, Facultad de Odontología, Universidad Nacional de Córdoba, Córdoba (Argentina), 30 June 1978

Summary. Arsenite but not oxamate produce in vitro a distinct depression of estrogen-dominated uterine motility, both in the absence of substrate as well as in the presence of exogenous glucose or lactate. The addition of oxamate to preparations suspended in a medium with lactate as the sole external substrate ameliorates the depression of uterine motility elicited by arsenite.

Oxytocin-induced contractions of isolated rat uterus under estrogenic dominance are influenced by the kind of metabolic substrate present in the suspending medium[4]. In a substrate-free solution, uterine contractions failed progressively following isolation and mounting. Glucose or lactate was able to prevent this decrement, whereas pyruvate produced a greater deterioration of contractile activity than that seen after the mere removal of substrate[4].

Enzyme inhibitors also influenced the in vitro motility of the rat uterus[4,6]. Indeed, 2-deoxy-D-glucose, a blocker of anaerobic glycolysis, elicited a distinct decrement of uterine motility when added to preparations kept in a glucose-containing solution[4]. On the contrary, oxamate, a potent inhibitor of the lactate-dehydrogenase (LDH) system, is devoid of influence on the functional activity of uterine horns isolated from rats under estrogenic dominance and kept in a lactate-containing solution[5]. This failure to alter contractions occurs despite a significant decrement in tissue pyruvate formation[5].

In order to shed some light on motility changes of uterine smooth muscle fibers exposed to glucose, lactate or pyruvate as well as about the lack of contractile of influences of oxamate; the effect of arsenite, an inhibitor of the entrance of pyruvate into the Krebs cycle[7], on the oxytocin-induced functional activity of isolated rat uterine horns, was explored.

Methods. Female Wistar rats, weighing between 150 and 200 g, were used. All the animals were injected i.p., during the anestrus, with a single dose of estilbestrol (1 µg/100 g, b.wt) and 17 h later decapitations was performed. Forthwith, strips of uterine horns were excised and suspended in a tissue chamber filled with 20 ml of Krebs-Ringer bicarbonate solution[4], gassed with 95% O_2+5% CO_2 and maintained at 37 °C and pH 7.4. This solution had either no substrate or was additioned with glucose, Na-lactate or Na-pyruvate at 11.0 mM. Possible effects of increasing osmolarity of the medium arising from the presence of substrates, were discarded after assessing the influences elicited by sucrose. Horn ends were then attached to a glass holder and to a strain gauge, respectively, subjected to a resting tension of 500 mg by means of a micrometric device and explored for contractile activity with the aid of a direct writing oscillograph. Values of isometric developed tension (IDT, in mg) and of frequency of contractions (FC, number of contractile cycles every 10 min) recorded at a constant resting tension, were measured in order to calculate the functional activity (FA, expressed in arbitrary units) as proposed elsewhere[4,6,8].

Uterine horns were allowed to equilibrate during a period of 1 h at the end of which most preparations had became quiescent. Postequilibrium noncontracting uterine horns were stimulated with oxytocin (Syntocinon®, Sandoz) at 0.25 mU/ml (final concentration in the bath solution). Preparations still active following equilibration period were discarded. Initial controls of the oxytocin-induced FA were taken and 10 min later Na-oxamate (at 10 mM) or Na-arsenite (at 0.1 or 0.3 mM) were delivered to the bath solution of some experimental preparations, whereas others remained as untreated controls.

No osmotic effect of Na-oxamate was detected, as shown by tests made with equiosmolar concentrations of sucrose. The activity of treated and untreated preparations was then followed during a period of 90 min. Variations in FA, against initial control values, were calculated and expressed as percent changes. Differences between experimental groups were considered significant at $p = 0.05$ or less.

Results and discussion. The results obtained are summarized in the table. Arsenite decremented FA levels of preparations kept in the absence of exogenous substrate. Inasmuch as in a substrate-free medium, the magnitude of FA is mainly determined by the availability of metabolic endogenous energy sources of muscle fibres, the expected blockade of the pyruvate-dehydrogenase system following arsenite, presumably enhanced the concentration of tissue pyruvate, a factor which has been proved to depress uterine motility through an impairment of carbohydrate breakdown[4,9]. The finding that a similar influence of arsenite was observed when pyruvate is the sole exogenous substrate, favours the notion that the contractile depression produced by this metabolite is directly associated to its presence rather than to a consequence of increased concentrations of products of its metabolism in the Krebs cycle.

The levels of uterine FA were significantly better in glucose or in lactate than in pyruvate-containing medium. The addition of arsenite depressed motility of preparations maintained both in glucose or in lactate solution; however, its influence was less marked in the first case. It is plausible

Effects of metabolic substrates and enzyme inhibitors over the oxytocin-induced funcational activity of uterine horns isolated from estrogen-dominated rats*

Substrate (mM)	Enzyme inhibitor (mM)	Functional activity (percent of changes at 90 min following equilibrium)**
None	None	−52.3±5.2 (15)
None	Arsenite 0.1	−80.4±6.3 (6)
None	Arsenite 0.3	−94.4±4.6 (6)
Pyruvate 11.0	None	−71.6±8.3 (9)
Pyruvate 11.0	Arsenite 0.1	−89.7±4.5 (6)
Pyruvate 11.0	Arsenite 0.3	−97.2±2.6 (6)
Glucose 11.0	None	−23.4±6.4 (12)
Glucose 11.0	Arsenite 0.1	−76.5±3.3 (9)
Glucose 11.0	Arsenite 0.3	−87.5±2.1 (7)
Lactate 11.0	None	−29.3±4.3 (9)
Lactate 11.0	Arsenite 0.1	−91.2±2.5 (12)
Lactate 11.0	Arsenite 0.3	−98.6±1.8 (6)
Lactate 11.0	Oxamate 10.0	−27.7±5.3 (10)
Lactate 11.0	Oxamate 10.0 + Arsenite 0.1	−81.9±2.1 (11)

* Means±SEM. Figures between parentheses refer to the number of preparations. ** FA = IDT × FC. For conditions and details see text.

that the energy yield from glucose to lactate, not affected by arsenite, may have some bearing on this difference.

The table shows that oxamate fails to alter the FA of uterine horns isolated from estrogen-dominated rats and maintained in a lactate-containing medium. This situation is in keeping with findings of previous experiments[5].

Although we do not have direct evidences that, under estrogen dominance and blockade of LDH system, lactate may be metabolized by the rat uterus via a pathway by passing pyruvate formation[5]; there exist observations indicating that, in several tissues exposed to low oxygen tension, lactate is directly converted into fumarate[10].

It has been shown in the rat uterus suspended in a medium with lactate that the presence of oxamate plus arsenite can block the formation of pyruvate from lactate as well as the entrance of pyruvate into the Krebs cycle[5,7].

However, the FA of estrogen dominated uterine horns isolated in lactate media and exposed to the combination of both agents, is significantly less reduced ($p < 0.02$) than when arsenite was the sole enzyme inhibitor, i.e. the presence of oxamate ameliorates the impairment of uterine motility following arsenite. This puzzling finding is also compatible with the possibility for the estrogenized rat uterus to obtain, at least under certain conditions, some contractile energy from lactate metabolized by alternative metabolic reactions, other than the formation of pyruvate, as has been documented to be the case in vascular smooth muscle preparations[11].

1 This work has been supported by grants 6638 and 6294 from the Consejo Nacional de Investigaciones Científicas y Técnicas de la República Argentina.
2 Senior Investigator of the Consejo Nacional de Investigaciones Científicas y Técnicas de la República Argentina.
3 Junior Investigator of the Consejo Nacional de Investigaciones Científicas y Técnicas de la República Argentina.
4 A. Goldraij, A.L. Gimeno and M.F. Gimeno, Acta physiol. latinoam. *23*, 113 (1973).
5 A.L. Gimeno, A. Goldraij, M.F. Gimeno and R. Santillán de Torres, Reproducción *3*, 5 (1976).
6 A. Goldraij, A.L. Gimeno and M.F. Gimeno, Acta physiol. latinoam. *25*, 229 (1975).
7 J.L. Webb, in: Enzyme and Metabolic Inhibitors, vol. III. Academic Press, New York 1966.
8 A. Goldraij, A.L. Gimeno, J.F. Vastik and M.F. Gimeno, Reproducción *1*, 31 (1975).
9 E.A. Newsholme, P.J. Randle and K.L. Manchester, Nature. Lond. *193*, 270 (1962).
10 E. Mensen De Silva and A. Cazorla, Am. J. Physiol. *224*, 669 (1973).
11 B.M. Altura and B.T. Altura, Am. J. Physiol. *219*, 1698 (1970).

Presence of sodium transport inhibiting factor in dog plasma during volume expansion[1]

R.H. Sterns, I. Singer and P.U. Feig

Department of Medicine, University of Pennsylvania, School of Medicine and Veterans Administration Hospital, Philadelphia (Pennsylvania 19104, USA), 10 July 1978

Summary. Plasma dialysates from volume-expanded dogs (E) were compared directly to dialysates from the same dogs when hydropenic. In a double-blind study, E caused relative inhibition of short-circuit current in toad urinary bladder. We therefore confirm the presence of a sodium transport inhibiting factor in plasma of volume-expanded dogs.

The search for a natriuretic hormone associated with volume expansion has been recently reviewed[2,3]. Due to the multiple variables involved in natriuresis in vivo[4], an effort has been made to develop in vitro bioassays for the hormone. After earlier experiments showing inhibition of sodium transport when frog skin was incorporated into the circulation of volume-expanded dogs[5], isolation of an antinatriferic factor was attempted using the anuran membrane for assay[6–8]. Buckalew et al. showed that dialysates of plasma from volume-expanded dogs inhibit sodium transport, as measured by short-circuit current (SCC)[9] in toad urinary bladder (TUB) and frog skin[6,10], but these studies could be criticized on several grounds: a) volume-expanded and control dialysate were from *different animals;* b) control animals were treated with furosemide, which was *assumed* to have negligible effects on the assay; c) assays were not performed on paired quarter-bladders, a model which offers better control against biological variability in the assay system; d) while the antinatriferic effect may be directly related to membrane resistance[11], low resistance membranes were not necessarily discarded; and e) assays were not 'double-blind'.

Other authors[2,12,13] were unable to confirm Buckalew's results, possibly because of one or more of the above factors. Moreover, preliminary data by Buckalew suggested that antinatriferic effect could be inhibited by diffusion of potassium from the electrical bridges into the bathing media[14]. The present study attempts to eliminate the problems listed above. Under 'double-blind' conditions, plasma dialysates obtained from volume-expanded dogs (E) were compared directly to plasma dialysates obtained from the same dogs when hydropenic (H). The comparisons were rigorously controlled in paired quarter-bladders, rejecting membranes of low resistance in advance, and specifically examining the effects of changes in medium potassium concentration.

Methods. 4 female mongrel dogs were studied twice each in alternating sequence with 2 weeks interval between studies. For one study, each dog, previously on ad libitum food and water, was anesthetized with pentobarbital sodium (30 mg/kg) and given an i.v. infusion of 1200 to 1500 ml of 0.9% saline solution over 90 to 120 min; at the end of the infusion, 75–100 ml of blood were obtained from the jugular vein. In the other study, each dog was deprived of sodium and water for 24 h, anesthesia was induced in the same manner and after 90 to 120 min without infusion (as a time control) blood was obtained, as above. Plasma ultrafiltrates were prepared with Diaflo PM-10 membranes (American Corp. Lexington, Mass.) with a mol. wt cutoff of 10,000[15].

For the bioassay, urinary bladders were removed from doubly-pithed female toads from the Dominican Republic (National Reagents, Inc., Bridgeport, Conn.). Half-bladders were placed in double lucite chambers with a 2.7 cm^2 cross-sectional area for each channel. Bladders with a SCC less than 20 μA, with a resistance less than 1000 Ω/cm^2 or less than 20% match between 2 contiguous quarter-bladders were discarded prior to the bioassay. (Mean resistance of bladders used was 2000 Ω/cm^2.) The bathing media contained (in mM): Na^+ 115, K^+ 3.5, Cl^- 95, HCO_3^- 25, Ca^{++} 1.0, $PO_4^{=}$ 0.5. For the studies on the effect of increasing K^+-concentrations, K^+ was 7.0 mM and Cl^-