J. Pharm. Pharmacol. 1981, 33: 604–606
Communicated December 22, 1980

0022–3573/81/090604–03 $02.50/0

The action of $PGF_{2\alpha}$ and oxytocin on rat and guinea-pig isolated uterus in the presence of a glucose metabolism stimulator: fructose-1,6-diphosphate††

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Several studies on rat adipose tissue (Prosdocimi et al 1979) have shown fructose-1,6-diphosphate (FDP) to potentiate the lipolytic action of catecholamines. Such an effect is probably correlated with the capability of FDP to stimulate the glycolytic process (Kirtley & McKay 1977) as well as to increase the availability of intracellular ATP and cAMP (Prosdocimi et al 1979). Since the cyclic nucleotides, cAMP, cGMP, have a role in regulating smooth muscle function (Polacek et al 1971; Diamond & Holmes 1975; Nesheim & Sigurdsson 1978) we have attempted to verify whether FDP would have some effect in particular on uterine smooth muscle.

We wanted to see (i) if FDP could cause rat and guinea-pig uterus fragments previously hypercontracted by $PGF_{2\alpha}$ or oxytocin or ergotamine to prolapse, and (ii) whether FDP was able to potentiate subliminar doses of hormone or autacoid.

Materials and methods

Female Wistar rats (Morini farm), 200–250 g, and female guinea-pigs, 350 g (bred in our Institute), were kept under standard conditions of humidity and temperature, and fed a standard laboratory diet (Mignini, Perugia). Non-pregnant animals were treated s.c. with 0·1 mg kg^{-1} diethylstilboestrol 24 h before an experiment; the pregnant animals were similarly treated but used only during the first 10 days of pregnancy. After 12 h starvation, animals were killed by cervical dislocation and carotid section. Both uterine cornua were removed according to Levy et al (1963). Uterine segments were suspended in Tyrode's solution aerated with a 5% CO_2 in oxygen. The effect of the compounds on uterine motility was recorded isotonically on a kymograph after 30 min equilibration.

Materials. Fructose-1,6-diphosphate sodium salt (Biomedica Foscama, Roma); oxytocin (Sandoz, Milan); ergotamine (Sandoz, Milano); prostaglandine $F_{2\alpha}$ (Upjohn, Milan); diethylstilboestrol (Maggioni, Milan). All other compounds came from standard suppliers.

Results

The effect of cumulative doses of oxytocin and $PGF_{2\alpha}$ was evaluated on uteri from pregnant rats and from diethylstilboestrol-primed rats. From the dose vs action curves so obtained, subliminar doses (max ineffective concentration) and minimal fully active doses (concentrations causing hypercontracture) where chosen for each compound.

Subliminar doses were respectively 10^{-12} M for $PGF_{2\alpha}$ and 10^{-6} I.U. ml^{-1} for oxytocin, while those causing

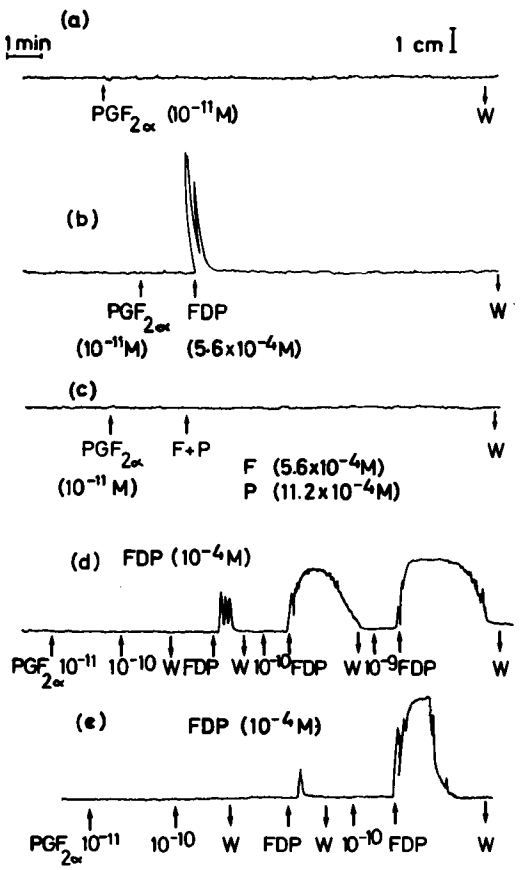

FIG. 1 (a) Effect of $PGF_{2\alpha}$ (10^{-11} M) alone; (b) effect with FDP; (c) with fructose (F) + phosphate (P) in rat uterus pretreated with diethylstilboestrol; (d) effect of FDP on non-effective doses of $PGF_{2\alpha}$ (10^{-10} and 10^{-9} M) in guinea-pig pregnant uterus and (e) guinea-pig non-pregnant uterus.

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†† This study was supported by a grant from Consiglio Nazionale delle Ricerche, C.N.R., Grant (CT) n. 79.01884.04.

hypercontracture were 10^{-6} M for $PGF_{2\alpha}$ and 10^{-3} I.U. ml^{-1} for oxytocin. Such doses remained in the same order of magnitude whether the preparation was rat or guinea-pig pregnant uterus, or oestrogen-primed rat uterus.

The effect of FDP was then evaluated on the uterine muscle response to such doses. In every experiment, the effect of FDP was also compared with the one induced by fructose + phosphate (F + P) at molar concentrations corresponding to those of FDP.

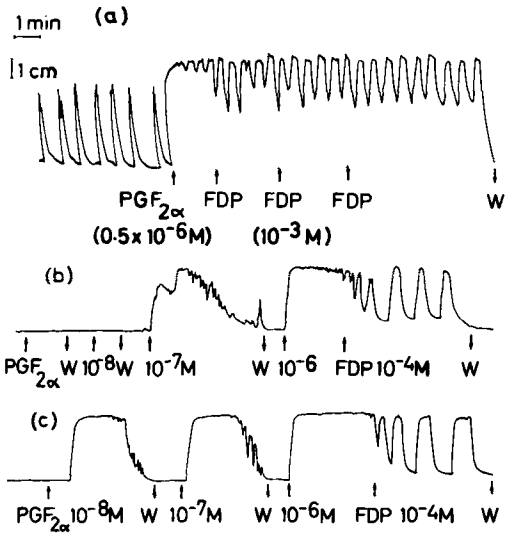

FIG. 2a. Effect of FDP on hypercontractive dose of $PGF_{2\alpha}$, (a) ($0{\cdot}5 \times 10^{-6}$ M) in rat uterus pretreated with diethylstilboestrol, (b) (10^{-6} M) in guinea-pig non pregnant uterus, (c) (10^{-6} M) in guinea-pig pregnant uterus.

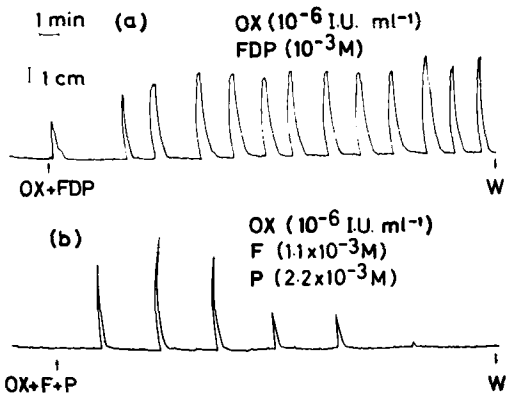

FIG. 3 (a) Effect of FDP on non-effective dose of oxytocin (10^{-6} I.U. ml^{-1}) in rat uterus pretreated with diethylstilboestrol. (b) Effect of fructose (F) + phosphate (P) on non-effective doses of oxytocin (OX, 10^{-6} I.U. ml^{-1}) in rat uterus pretreated with diethylstilboestrol.

Effect of FDP on the response induced by subliminar or hypercontracting doses of $PGF_{2\alpha}$. Both in oestrogen-primed rat uterus and in pregnant guinea-pig uterus, FDP ($5{\cdot}6 \times 10^{-4}$ M) was inactive by itself, but it caused a clear contractile response when added subsequently to ineffective doses of $PGF_{2\alpha}$ (Fig. 1a,b,d,e).

Molar doses of F + P corresponding to those of FDP did not evoke any contractile response (Fig. 1c).

In preparations from diethylstilboestrol-primed rats and from pregnant or non-pregnant guinea-pigs, FDP (10^{-3} M) added after doses of $PGF_{2\alpha}$ causing hypercontraction, not only suppressed the spastic condition, but also evoked a clearly rhythmic activity, which was maintained (Fig. 2a,b,c).

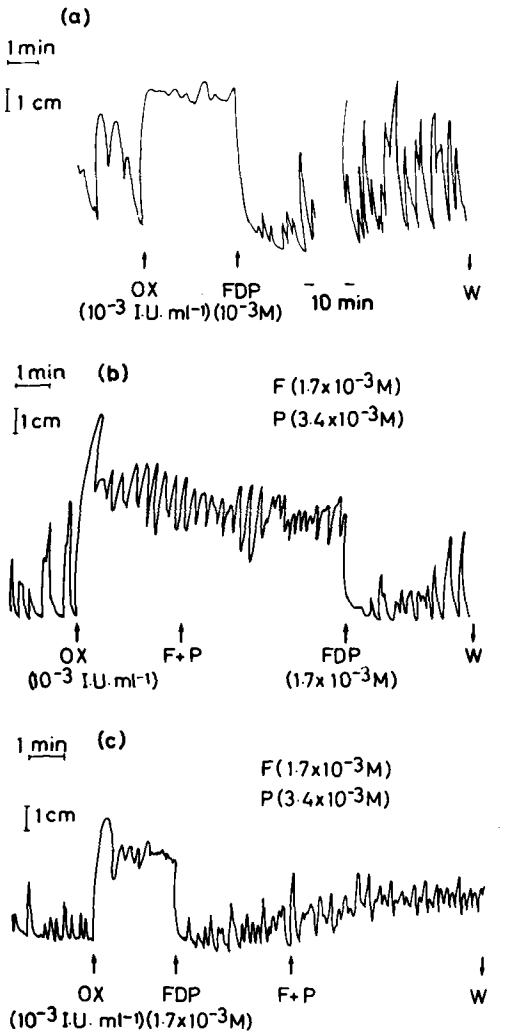

FIG. 4. Effect on a hypercontractive dose of oxytocin (10^{-3} I.U. ml^{-1}) of FDP (a) without, (b) with equimolar fructose (F) + phosphate (P) in pregnant rat uterus, (c) fructose (F) + phosphate (P) and equimolar FDP in pregnant rat uterus.

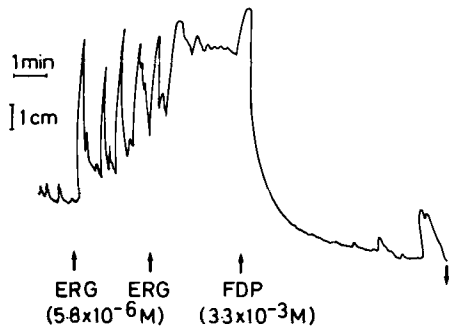

FIG. 5. Effect FDP on hypercontractive dose of ergotamine ($5{\cdot}8 \times 10^{-6}$ M) in pregnant rat uterus.

Effect of FDP on responses induced by subliminar or hypercontracting doses of oxytocin. In a manner similar to the induced effects on the uterine response to $PGF_{2\alpha}$, FDP (10^{-3} M) induced a rhythmic activity even in the uterus (diethylstilboestrol-primed rat) pretreated with subliminar doses of oxytocin (Fig. 3a). In the same preparation, F + P (in doses corresponding to those of FDP) induced only a temporary rhythmic activity, which faded quickly (Fig. 3b). In pregnant rat uterus, FDP (10^{-3} M) induced a clear and immediate prolapse in preparations hypercontracted by addition of 10^{-3} I.U. ml^{-1} oxytocin. As in the case of $PGF_{2\alpha}$, even in presence of doses of oxytocin causing hypercontraction, FDP not only suppressed the spastic condition, but it also allowed the reappearance of a regular rhythmic activity (Fig. 4a).

There was a difference in behaviour between FDP and F + P. The latter, cannot antagonize a contraction induced by oxytocin (Fig. 4b), while a subsequent addition of FDP to the same preparation is able to fully restore the rhythmic activity (Fig. 4b). Moreover, while FDP resolves the condition of tetanic contraction induced by the hormone, F + P added subsequently to the same preparation are either inactive or tend to reset the oxytocin-induced hypertone (Fig. 4c).

Effect of FDP on the response induced by doses of ergotamine causing hypercontraction. With the aim of extending our survey on the influence of FDP on the response of uterine muscle to oxytocic compounds, the effect of FDP in hypercontraction produced by ergotamine was evaluated on rat pregnant uterus.

FDP also antagonizes the hypercontraction induced by ergotamine ($5{\cdot}8 \times 10^{-6}$ M), thus confirming the ability of FDP to control the response of smooth muscle fibre to stimuli causing contracture (Fig. 5).

Discussion

The data reported indicate that in rat uterus (either pregnant or not, either oestrogen primed or not), FDP potentiates ineffective doses of $PGF_{2\alpha}$ and oxytocin, while it antagonizes the hypercontraction induced not only by the above compounds, but also that by ergotamine, reducing the tonic phase and bringing about a persistent rhythmic contraction.

This fact is in contrast with the inability of F + P to modify the responses of preparations to any of the stimulants (oxytocin and $PGF_{2\alpha}$). FDP seems to be a compound capable of modulating uterine smooth muscle responses to hormonal and pharmacological stimuli, because it amplifies the effects of subliminar stimuli, but it also reduces those caused by excessively severe stimuli. The mechanism of action of FDP in the events described is still unsolved.

Studies on other biological substrates, such as adipose tissue (Prosdocimi et al 1979), heart (Markov et al 1978, 1979), and blood (Magalini et al 1977; Perroni et al 1980) have shown FDP to be capable of influencing cellular energy metabolism. In particular, FDP increases intracellular ATP and cAMP levels (Prosdocimi et al 1979), and positively influences glycolytic enzymes (Kirtley & McKay 1977). Under conditions of subliminar stimuli, an increase in the energy availability might promote the onset of contractile responses, as shown by Bülbring et al (1968) in guinea-pig uterus. Under hypercontraction, on the contrary, ionic movements (Cattani et al 1980), together with variations in cAMP level (Prosdocimi et al 1979) might play a role mainly in the relaxing effect of FDP. The speed of the FDP anti-contraction effect might perhaps indicate that it acts by affecting ionic movements.

REFERENCES

Bülbring, E., Casteels, R., Kuriyama, H. (1968) Br. J. Pharmacol. 34: 388–407

Cattani, L., Costrini, R., Cerilli, C., Rigobello, M. P., Bianchi, M., Galzigna, L. (1980) Agressologie 21: 263–264

Diamond, J., Holmes, T. G. (1975) Can J. Physiol. Pharmacol. 53: 1099–1107

Kirtley, M. E., McKay, M. (1977) Mol. Cell. Biochem. 18: 141–149.

Levy, B., Tozzi, S. (1963) J. Pharmacol. Exp. Ther. 142: 178–184

Magalini, S. I., Bondoli, A., Scrascia, E. (1977) Resuscitation 5: 103–110

Markov, A. K., Chilliard, N., Lehan, P., Hellems, H. (1978) Circulation: 57–58 Suppl. II (n. 379)

Markov, A. K., Oglethorpe, N., Young, D. B., Lehan, P. H., Hellems, H. K. (1979) Clinical Research (Meeting of American Society for Clinical Investigation), Abs 27: 440A

Nesheim, B. I., Sigurdsson, S. B. (1978) Acta Pharmacol. Toxicol. 42: 371–376

Perroni, L., Ensoli, G., Nunziata, A., Soscia, F., Costrini, R., Gioia, N., Perri, G. C. (1980) Pharmacol. Res. Commun. 12: 147–153

Polacek, I., Bolan, J., Daniel, E. E. (1971) Can. J. Physiol. Pharmacol. 49: 999–1004

Prosdocimi, M., Caparrotta, L., Dorigo, P., Fassina, G., Siliprandi, N. (1979) Biochem. Pharmacol. 28: 281–285