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Dilinoleyl lecithin
labor
methyl arachidonate
methyl linoleate
oxytocin
phospholipids

Phospholipids and Fatty Acids in Relation to the Premature Induction of Labor in Rabbits

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Extract

Additional lipids have been tested to determine whether they share with soya bean phospholipids (SBP) and dilinoleyl lecithin (DLL) the ability to sensitize the pregnant rabbit uterus to oxytocin, which permits the induction of premature labor at a time otherwise not possible. Comparable activity was given by none of the compounds tested intravenously but was given by methyl linoleate and methyl arachidonate administered by the intrauterine route, both at a dose of 1 g; abortion rates for these compounds were 69% ± 4.3% and 79% ± 7.8%, respectively. These values do not differ significantly from those for intravenous SBP and DLL.

Speculation

All highly active compounds thus far found have either linoleate or arachidonate as a part of their molecule. These essential fatty acids are precursors of prostaglandin E or $F_{2\alpha}$, both of which can stimulate myometrial contractions in the pregnant rabbit uterus and cause luteolysis. We postulate that active lipids act by conversion to prostaglandins, which in turn prepare the uterus for delivery. Both the time interval required to achieve this effect and the ability of progesterone to block it suggest that the prostaglandins act by luteolysis rather than by stimulation of myometrial contractions.

Introduction

In 1963 Luukkainen and Csapo [9] reported that intravenous administration of a preparation of SBP contained in Lipomul, an intravenous fat preparation, altered the response of the pregnant rabbit uterus to oxytocin so that premature labor could be induced. The pregnant rabbit uterus normally remains relatively unresponsive to oxytocin until about *day 29* of pregnancy; delivery usually occurs on *day 30–32*. After SBP administration, however, labor can be induced with oxytocin as early as *day 21*.

We [11] have previously characterized the nature of the active material in SBP and believe that it is attributable largely to linoleic (18/2) acid as a component of lecithin. Synthetic dilinoleyl lecithin in appropriate dosage reproduced the biologic activity of SBP. We wondered whether other lipids or fatty acids could be found that were also biologically active, and have tested a few. The results suggested a relation between biologic activity and two of the essential fatty acids, linoleic and arachidonic (20/4) acid. Both of these are "essential" fatty acids, which are precursors of prostaglandins, with pronounced effects on the gravid uterus and on the corpus luteum.

Materials and Methods

Biologic testing was carried out in 25-day pregnant rabbits, by intravenous or intrauterine infusion, the latter

Table I. Fatty acid composition of test compounds[1]

Compound	Formula	SBP	DLL	Triglycerides			Me esters							Ethyl ester,	Free acid,
				16/1	18/1	18/2	10/0	18/0	18/1	18/2	18/3	20/0	20/4	18/2	18/0
Capric	10/0						99.2								
Lauric	12/0	0.3													
Myristic	14/0	0.2	1.4				0.3					0.4			
Palmitic	16/0	15.1	0.6		0.2							1.1			
Palmitoleic	16/1		0.3	97.2	0.2										
Palmitlinoleic	16/2				0.2	2.2									
Stearic	8/0	3.7	0.3					100							100
Oleic	18/1	14.0	1.2		99.2	0.8			100		0.5			1.9	
Linoleic	18/2	60.1	93.4	2.8		92.0				100	1.7			94.0	
Linolenic	18/3	6.6	0.8			0.7					97.8				
Arachidic	20/0											98.5		0.8	
Eicosenoic	20/1														
Arachidonic	20/4					0.4							97.4		
Behenic	22/0		0.3												
Erucic	22/1												2.6		
Unknown			1.7		0.2	3.7	0.5							3.3	

[1] Values are expressed as weight percent; limit of sensitivity was 0.1%; SBP: soya bean protein; DLL: dilinoleyl lecithin.

via a plastic catheter inserted at laparotomy into the uterus near the entry of the fallopian tube. Test lipids were assayed for purity by lipid class and fatty acid analysis: the former by thin layer chromatography [14], and the latter by gas-liquid chromatography with a hydrogen flame ionization detector after methylation with 14% BF_3-methanol as described by Morrison and Smith [10]. Results of these analyses together with an analysis of SBP are shown in Table I. For infusion, test materials were sonicated in the cold in 40 cu cm of 25% rabbit serum in 5% dextrose. The resulting emulsion was given by the intrauterine route; for intravenous use it was further diluted to 250 cu cm with 5% dextrose. Infusions were given over 4–5 hr. At 24, 48, and 49 hr after infusion, 1 unit of oxytocin was given intramuscularly and the rabbit was observed for abortion. One to 2 hr after the last injection of oxytocin, the rabbit was killed and autopsied. The number of fetuses aborted was expressed as a percentage of the total number in the pregnancy. A minimum of five rabbits was tested for each compound, with controls interspersed by random numbers. Controls were identically treated with solutions of sonicated rabbit serum and 5% dextrose. Significance testing was by chi-square analysis.

Results

For compounds tested by the intravenous route (Table II), only dilinoleyl lecithin was found to reproduce the high abortion rates, usually 65% or more, found on testing with SBP. Trilinolein yielded an abortion rate significantly higher than in controls, but in an intermediate range significantly lower than that for SBP. Toxicity precluded testing trilinolein at a higher dose. Ethyl linoleate by the intravenous route was inactive.

By the intrauterine route, SBP, our reference compound for activity by the intravenous route, proved inactive, but methyl linoleate at a dose of 1 g yielded the high abortion rates (over 65%) encountered when testing SBP intravenously. Of other fatty acid methyl esters tested at the same dose, arachidonic (20/4) acid was also highly active. Three other methyl esters, stearate (18/0), oleate (18/1), and arachidate (20/0), gave results in an intermediate range, significantly higher than controls, but significantly lower than for linoleate and arachidonate.

Discussion

Previous work [11] has shown that the biologic activity of SBP lay in its choline-containing, predominantly lecithin fraction; the predominant fatty acid of this fraction was linoleic acid, and saturation by hydrogenation destroyed biologic activity. Synthetic DLL in appropriate dosage reproduced the activity of SBP [8].

Testing by the intravenous route of the additional compounds shown in Table II failed to reveal any with the activity of SBP or DLL. A questionable exception was trilinolein, but repeated testing at the highest dose tolerated failed to show more than a modest activity, significantly greater than control values but also significantly below values for SBP and DLL. Ethyl linoleate was entirely inactive, as is perhaps consistent with

Table II. Abortion rates in rabbits after administration of various lipids[1]

Material	Route	Dose/ rabbit, g	Total no. fetuses	Aborted, % (±SE)
Soya bean phospholipids[2]	i.v.	2.0	106	83 ± 3.6[3]
Soya bean phospholipids[2]	i.v.	4.0	90	90 ± 3.2[3]
Dilinoleyl lecithin[2]	i.v.	1.0	51	57 ± 6.9[3]
Dilinoleyl lecithin[2]	i.v.	2.0	31	100 ± 0[3]
Dipalmitoyl lecithin[2]	i.v.	2.0	23	19 ± 6.9
Tripalmitolein	i.v.	1.5	41	5 ± 3.4
Triolein	i.v.	1.0	26	0 ± 0
Trilinolein	i.v.	1.0	70	37 ± 5.8[3]
Methyl decanoate	i.v.	0.4	41	0 ± 0
Stearic acid	i.v.	0.4	43	12 ± 4.9
Ethyl linoleate	i.v.	1.5	29	0 ± 0
Controls	i.v.		401	6 ± 1.2
Soya bean phospholipids	i.u.	4.0	37	0 ± 0
Soya bean phospholipids	i.u.	3.3	43	7 ± 3.9
Methyl decanoate	i.u.	1.0	53	2 ± 2.1
Methyl stearate	i.u.	1.0	69	36 ± 5.8[3]
Methyl oleate	i.u.	1.0	34	27 ± 7.6[3]
Methyl linoleate	i.u.	1.0	113	69 ± 4.3[3]
Methyl linolenate	i.u.	1.0	47	19 ± 5.7[4]
Methyl arachidate	i.u.	1.0	41	2 ± 2.4
Methyl arachidonate	i.u.	1.0	28	79 ± 7.8[3]
Controls	i.u.		257	8 ± 1.7

[1] Test material was infused into 25-day pregnant rabbits. Oxytocin was injected on *days 26* and *27*.
[2] From previous experiments (8, 11).
[3] $P < 0.01$ *versus* controls.
[4] $P < 0.05$ *versus* controls.

the extremely short biologic half-life of intravenously administered fatty acids.

Most of the compounds tested intravenously were insoluble, and some were toxic. Doses were the highest attainable within these limits, with the exception of methyl decanoate and stearic acid, which were given in doses said to be capable of inducing myometrial sensitization to oxytocin in the pregnant rabbit uterus [7]. Our results do not confirm this report.

Intrauterine testing revealed two significant findings initially; SBP, our reference material for intravenous testing, was not active, but methyl linoleate was active at a dose of 1 g. Several other fatty acid esters were tested at the same dose, and only one, arachidonate, proved highly active. Three others showed an intermediate and questionable activity; as with intravenous trilinolein, the results were significantly above control values but significantly below those for intrauterine methyl linoleate or intravenous SBP or DLL. In the case of stearate, the modest activity shown by the fatty acid ester given by the intrauterine route was not matched by corresponding activity of hydrogenated SBP given intravenously. Hydrogenation of SBP converted its fatty acids largely to stearate without demonstrably changing its composition by lipid class analysis [11]. The high percentage of linoleate (58%) carried as a component of lecithin in SBP was replaced by an even higher percentage of stearate (87%), also carried as a component of lecithin, and at the same time biologic activity was destroyed. The observation leads us to believe that the moderate activity displayed by intrauterine stearate may reflect a different mode of action from that associated with intravenous SBP or intrauterine methyl linoleate.

Thus far, the highly active compounds which we have found have been restricted to lecithin given intravenously with linoleate as the predominant (SBP) or virtually sole (DLL) fatty acid, and to methyl linoleate or methyl arachidonate esters given by the intrauterine route. We believe that the role of phospholipid by the intravenous route is to act as a transport vehicle to deliver an appropriate fatty acid to its target, presumably the uterus; phospholipids have a relatively long half-life in the circulation as opposed to the very short half-life of fatty acids. The inactivity of SBP given by the intrauterine route might reflect an absorption barrier or the lack of an appropriate intrauterine lecithinase to split off the α or β fatty acid. Both methyl linoleate and methyl arachidonate esters were active when given by the intrauterine route. Both are precursors of prostaglandin E_2 and $F_{2\alpha}$ [13], and may act by conversion to prostaglandins. Prostaglandin E_2 and $F_{2\alpha}$ could, in turn, act in two ways: (*1*) They could stimulate myometrial contractions, directly. Both prostaglandins are highly effective in stimulating such activity in the pregnant uterus [3, 4]. (*2*) They could cause corpus luteum involution. Both prostaglandins are also active in this role in the rabbit [12].

The second action appears more likely. Lipid infusion requires 24–48 hr to achieve the biological effect we are observing. The half-life of prostaglandins is very short [5, 6], and their action in stimulation of myometrial contractions is evanescent. However, the rabbit is entirely dependent for pregnancy maintenance on corpus luteum synthesis of progesterone, and progesterone withdrawal, either by ovariectomy or by discontinuance of progesterone therapy in previously ovariectomized pregnant rabbits, is followed by abortion in 24–48 hr. Luteolysis would presumably be followed by a similar time lag. It may also be relevant that the ac-

tion of SBP can be blocked by progesterone administration [9]. Linolenic acid (18/3), which was inactive in our test, is also an essential fatty acid and also a prostaglandin precursor, but of prostaglandin E_3 [1]. This less well studied prostaglandin is less active in certain test systems [2], but no data on its action on the pregnant uterus or on luteolysis are available.

Summary

Several lipids have been tested to determine whether additional compounds could be found which shared with intravenously administered SBP and DLL their ability to sensitize the pregnant rabbit uterus to oxytocin, permitting induction of labor prematurely. No such compounds were found on testing by the intravenous route, but by the intrauterine route, methyl linoleate and methyl arachidonate gave responses comparable with those given by intravenous SBP and DLL. Ethyl linoleate was inactive when given intravenously; we believe this reflects the rapid metabolism of fatty acids in the circulation. SBP was inactive by the intrauterine route, which suggested either an absorption barrier to the phospholipid or lack of an appropriate intrauterine lecithinase to split off the fatty acids.

Modest activity not comparable with that shown by intravenous SBP was found for some compounds; evidence is given that this may reflect some other mode of action.

We postulate that active phospholipids serve as transport molecules to carry an appropriate fatty acid to a target organ, presumably the uterus. Linoleate and arachidonate are the only fatty acids thus far found to be active. Both are essential fatty acids and both are known to be prostaglandin precursors yielding prostaglandin E and $F_{2\alpha}$. We believe that active lipids act by conversion of an appropriate fatty acid to one of these prostaglandins, both of which are capable of stimulating myometrial contractions in the pregnant rabbit uterus and of causing luteolysis. Time relation and the ability to block activity with progesterone suggest that luteolysis is the more likely possibility.

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