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COMPARISON BETWEEN INHIBITORY EFFECT OF MELATONIN AND INDOMETHACIN ON PROSTAGLANDIN IN PREGNANT MICE

(With 3 Tables & 2 Figs.)

By

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المقارنة بين التأثير المثبط للميلاتونين والاندوميثاسين على البروستاجلاندين في الفئران الحوامل

مصطفى جابر ، إمتثال مصطفى

أدى حقن الميلاتونين أو الاندوميثاسين في اليومين التاسع عشر والعشرين من الحمل في الفئران إلى تأخير ميعاد الولادة الطبيعية وإلى زيادة ذات دلالة معنوية في زمن الولادة وكان معدل الوفيات منخفضاً ومتوسط عدد المواليد طبيعياً ·عند حقن البروستاجلاندين $F_{2\alpha}$ في اليومين الخامس عشر والسادس عشر من الحمل تمت الولادة في اليوم الثامن عشر · بينما حقن الميلاتونين أو الاندوميثاسين بعد حقن البروستاجلاندين $F_{2\alpha}$ كانت الولادة في العشرين من الحمل وعدد المواليد الاحياء طبيعياً · وقد أظهر الفحص المجهرى لأجنة الحيوانات التى حقنت بالبروستاجلاندين $F_{2\alpha}$ في الأيام التاسع والعاشر والحادى عشر من الحمل وجود عدد قليل من الإنقسامات الخلوية · بينما أظهرت مقاطع أجنة الحيوانات المحقونة بالميلاتونين أو الاندوميثاسين بعد حقن البروستاجلاندين $F_{2\alpha}$ وجود عدد كبين من هذه الإنقسامات · كما أدى إضافة مادة الميلاتونين أو الاندوميثاسين إلى المحلول المحيط بالرحم المعزول للفئران إلى نقص ذا دلالة معنوية في مدى ومعدل الإنقباضات الرحمية · مما يدل على أن هذه المواد لها تأثير مضاد للتأثير المنبه لمادة البروستاجلاندين · ويمكن أن نستنتج من هذا البحث أن الميلاتونين له بعض التأثيرات المشابهة للاندوميثاسين على البروستاجلاندين ·

SUMMARY

Intraperitoneal injection of either melatonin or indomethacin on days 19 and 20 of pregnancy in mice led to a delay in the onset and significant prolongation in the duration of spontaneous labour. The incidence of foetal mortality was low and a non-significant change in the mean number of alive pups was obtained.

In animals treated with melatonin on days 15, 16 and 17 of pregnancy after prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), the day of labour was 20.88±0.23 and the mean number of pups was 6.88±0.81 (all were born alive). Day of labour after $PGF_{2\alpha}$ alone was 18.25±0.16 and mean number of pups was 6.5±0.63. A nearly similar effect to melatonin was obtained

in animals treated with indomethacin.

Histological examination of the embryos of mice treated with melatonin on days 9, 10 and 11 of pregnancy after $PGF_{2\alpha}$ showed many number of mitosis indicating inhibition of the abortive effect of prostaglandin. Sections of embryos of animals treated with indomethacin showed nearly similar picture to that of melatonin.

In vitro study: melatonin produced significant decrease in the amplitude and frequency of uterine contraction. Both melatonin and indomethacin antagonised the stimulatory effect of $PGF_{2\alpha}$ on uterine contractions.

From this study, it can be concluded that melatonin has some similar effects to that of indomethacin on prostaglandins.

INTRODUCTION

Melatonin has been reported to have many effect on the reproductive system. In rodents melatonin delayed vaginal opening (WURTMAN, et al. 1963), inhibited pregnant mare serum-induced ovulation (REITER and SORRENTION, 1971), inhibited compensatory ovarian hypertrophy (VAUGHAN, et al. 1972) and prologed the oestrus cycle (VAUGHAN, et al. 1976).

Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) was also been reported to exert an antifertility effect in dogs and rabbits (GUTKNECHT, et al. 1971), mice (LABHSETWAR, 1972 a) and rats (LABHSETWAR, 1972 b).

Indomethacin (PGs synthetase inhibitor) delays parturition in rats (AIKEN, 1972), primates (NOVY, et al. 1974) and hamsters (LAU, et al. 1975 a). HAMADA, et al. (1978) and HOLMES, et al. (1983) reported that indomethacin blocked ovulation.

The aim of this work is to study the effect of melatonin on the onset and duration of normal parturition in mice and to compare the effect of melatonin with that of indomethacin on prostaglandin $F_{2\alpha}$.

MATERIAL and METHODS

Adult female albino mice (Charles river strain), 3 months old were used in this study. Females were paired with fertile males and the day when the vaginal plug was detected was designated as day 1 of pregnancy (FINN and McLARIN, 1967).

The pregnant animals were divided into 13 groups, 8 animals each. Groups 1-4 were used to study the effect of melatonin (Kock-Light laboratories) on the duration and the onset of normal labour. Animals of these 4 groups were allowed to complete their pregnancy for 19 days. Group 1 was considered as a control. Animals of groups 2, 3 and 4 were intraperitoneally (IP) injected with 50, 100 ug melatonin and 100ug indomethacin respectively twice daily on days 19 and 20 of pregnancy. All animals

of these groups were left under observation and the time when parturition started was noted. This was judged by birth of the first pup. Births of alive and dead pups were recorded starting at 8 am on days 21 and the time of the onset of parturition was recorded from this point.

Groups 5-8 were used to study the effect of melatonin on premature labour. Animals of these groups were 15 days pregnant. They were injected IP with 100 ug prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$, lutalyse, upjohn Co.) on days 15, 16 and 17 to induce premature labour (LAU, et al. 1975 b). Group 5 was kept as a control group. Groups 6, 7 and 8 were treated with 50, 100 ug melatonin and 100 ug indomethacin (Liometacen Nile Co.), respectively, IP twice daily for the same 3 days (days 15, 16 and 17).

Groups 9-12 were used to study the effect of melatonin on abortion. Mice were 9 days pregnant. Animals of group 9 were considered as control group. Animals of groups 10, 11 and 12 were injected with $PGF_{2\alpha}$ (100 ug) on days 9, 10 and 11 of pregnancy. Groups 11 and 12 were injected with melatonin (100 ug) and indomethacin (100 ug) respectively IP twice daily for the same 3 days. All animals of these groups (Groups 9-12) were injected with colchicine (0.1 mg/mouse; FINN and BREDL, 1973) subscutaneously 5 hours before the time of sacrifaction (day 12 of pregnancy) to show cell morphology and mitosis of the embryos on histological examination.

Group 13 was used to study the effect of melatonin (100 ug), $PGF_{2\alpha}$ (100 ug) and indomethacin (100 ug) on the uterine contractions in vitro. Isolated segments of the uteri from 19 days pregnant mice were suspended in an organ bath (50 ml capacity) containing De Jalon solution (9.0 gm NaCl, 0.42 KCl, 0.06 gm $CaCl_2$, 0.50 gm $NaHCO_3$, and 0.50 gm glucose; all dissolved in one liter distalt water). Temperature of the bath was kept at 30°C and the bath was continuously gassed with O_2 containing 5% Co_2. Movements of the segment were recorded using physiograph-six E, M. Instrument (Co. Inc., Houston. Texas, Marcocompany). Student's T-test was used in the statistical analysis of the results.

RESULTS

Effect of melatonin on the onset and duration of normal parturition in mice (Table 1):

Injection of melatonin (100 or 200 ug/day) produced delay in the mean time of onset and significant increase ($P/ 0.001$) in the duration of normal labour. Melatonin in a dose of 100 ug/day caused the longest mean delay in the onset of parturition (18.75±1.15 h), the longest duration of parturition (66.13±4.03%) and the lowest decrease (7.69%) proportion of pups born dead.

Indomethacin treatment (200 ug/day) showed a delay in the mean time of onset and significant increase ($P/ 0.001$) in the duration of normal labour. The onset of labour delayed by 17.87±2.21 h. The duration of labour was increased by 28.0±2.79%. The incidence of Foetal mortality was 22.62%. A non-significant change in the mean number of pups was observed.

Effect of melatonin on premature labour induced by $PGF_{2\alpha}$ in mice (Table II):

The mean time for the onset of premature labour in mice treated with $PGF_{2\alpha}$ was 18.25±0.16, the mean number of pups delivered was 6.5±0.63% of which born dead.

In animals treated with melatonin in a dose of 100 ug/day after $PGF_{2\alpha}$, the day of parturition was 19.13±0.30 and the mean number of pups was 6.63±0.74 per mice among which 3.77% were dead. In animals treated with 200 ug/day melatonin after $PGF_{2\alpha}$ the day of labour was 20.88±0.23 and the mean number of pups was 6.88±0.81 (all were born alive).

In animals treated with indomethacin after $PGF_{2\alpha}$ the day of parturition was 20.0± 0.38 and the mean number of pups was 5.87±0.44 among which 10.73% were born deat.

Effect of melatonin on abortion (Fig. 1-4):

Histological examination of the embryos of mice treated with $PGF_{2\alpha}$ showed no mitosis with degenerated cells. The cells were irregular in shape, pale in colour with fragmented nuclei (Fig. 1 B).

In contrast to this picture, embryos of mice treated with melatonin after $PGF_{2\alpha}$ (Fig. 1 C) showed more or less similar picture to that of the control (Fig. 1 A). Many numbers of mitosis were present. The cells were regular in shape, deeply stained with centeral nuclei.

Section of embryos of animals treated with indomethacin (Fig. 1 d) showed nearly similar picture to that of melatonin treated and control animals.

Effect of melatonin on uterine contraction (Table III):

Melatonin in a dose of 100 ug produced significant decrease ($P/ 0.05$) in th frequency and amplitude ($P/ 0.01$) of uterine contractions. The frequency of contractions decreased by 35.48%. While the amplitude of the contractions decreased by 45.60% (Fig. 2 D).

$PGF_{2\alpha}$ in a dose of 100 ug produced significant increase ($P/ 0.01$) in the frequency and amplitude ($P/ 0.001$) of uterine contractions. The frequency of contractions increased by 46.52% while the amplitude of the contractions increased by 70.99% (Fig. 2 A).

Addition of melatonin after $PGF_{2\alpha}$ produced non-significant increase in the frequency and amplitude of uterine contractions. The frequency of contractions increased by 19.65% while the amplitude of contractions increased by 33.63% (Fig. 2 B).

Addition of indomethacin after $PGF_{2\alpha}$ produced non-significant decrease in the frequency and significant decrease ($P/ 0.05$) in the amplitude of uterine contractions. The frequency of contractions decreased by 17.04%. While the amplitude of contractions decreased by 38.13% (Fig. 2 C).

DISCUSSION

In this study, intraperitoneal injection of melatonin into pregnant mice during the last few days of gestation (days 19 and 20) delayed the time of onset and significantly prolonged the duration of normal parturition. Premature labour that induced in mice by IP injection of $PGF_{2\alpha}$ few days before the normal time of parturition (at 15-18 days of gestation) was prevented by melatonin treatment. Histological examination of the embryos of mice treated with melatonin after $PGF_{2\alpha}$ on 9th day of pregnancy showed many mitosis. Similar results were recorded using indomethacin.

The delayed effect of melatonin on the onset of normal labour and prolongation of its duration, as well as preventing abortion and premature labour can be explained by the inhibitory effect of melatonin on uterine contractions. In this study, melatonin found to produce significant decrease in the amplitude and frequency of uterine contraction. Also melatonin was found to antagonise the action of $PGF_{2\alpha}$ on uterine contractions.

Another possible role for the action of melatonin on the onset of labour is that melatonin may inhibit the synthesis and release of $PGF_{2\alpha}$. This suggestion is consistent with the finding of LEACH and THORBURN (1980) and PAWLIKOWSKI, et al. (1984) who found that melatonin inhibited PGs synthesis in a variety of in vitro systems at concentrations greater than 430 nM. LEACH, et al. (1982) showed that injection of melatonin into cisterna magna blocks both PGE rise and ovulation induced by cervical stimulation in rabbits. LEWINSKI and PAWLIKOWSKI (1986) found that melatonin alters $PGF_{2\alpha}$ synthesis.

It is clear from this study that melatonin has nearly the actions of indomethacin. Both compounds delayed the onset of parturition and prolonged its duration. Also, both inhibited the action of PGs in induction of abortion and premature labour and both decreased the amplitude and frequency of uterine contractions.

The similarity of actions of melatonin and indomethacin can be supported by investigators. KAMBERI (1973) and OJEDA, et al. (1975) found that intraventricular injection of indomethacin reduced LH release in responce to oestrogen and progesterone. OJEDA, et al. (1979) and LEACH and THORBURN (1980) found that both compounds inhibit the release of $PGF_{2\alpha}$ and thormboxane A_2 synthesis.

The similarity of actions of melatonin and indomethacin can be attributed to the similarity in their chemical structure. LEACH and THORBURN (1980) stated that the chemical structure of melatonin closely resembles that of indomethacin. KELLY, et al. (1984) found that N. acety 1-5 methoxy kynurenamine, a brain metabolite of melatonin, is a potent inhibitor of PG biosynthesis.

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Table (I)

Effect of melatonin on the onset and duration of parturition inmice

Treatment	Onset of parturition (h) (recorded from 8 am 20th day)		Duration of parturition		No. of dead pups	
Treatment	Mean	Time of dealy	Mean	Time of delay	Mean No. of pups delivered/mice	Mean	Percent
Control	7.38±0.57		157.5±1.42		6.25±0.53	0.0	0.0
Melatonin (100 ug/day)	26.37±1.48	18.99±1.15	41.88±5.67***	26.13±4.03	6.50±0.65	0.5±0.27	7.69
Melatonin (200 ug/day)	21.63±1.25	14.25±1.50	32.50±2.11***	16.75±1.36	5.50±0.57	1.0±0.33	18.18
Indomethacin	25.25±1.85	17.87±2.21	43.75±1.83***	28.0±2.79	6.63±0.71	1.50±0.38	22.62

Table (II)

Effect of melatonin on premature labour in mice

Treatment	Day of parturion	Mean No. of pups delivered/mice	No. of dead pups	
			Mean	Percent
$PGF_2\alpha$	18.25±0.16	6.5±0.63	0.50±0.27	7.69
PGF_2 Mel (100 ug/day)	19.13±0.23*	6.63±0.81	0.25	3.77
PGF_2 + Mel. (200 ug/day)	20.88±0.23***	6.88±0.81	0.0	0.0
PGF_2 + Ind.	20.00±0.38***	687±0.44	0.63±0.26	10.73

Table (III)
Effect of melatonin on uterine contractions in mice

	Frequency		Amplitude	
	Rate/min	% Change	Hight in cm	% Change
Control	7.75±0.88		3.64±0.40	
Melatonin	5.00±0.33*	35.48	1.98±0.14**	45.60
Control	6.75±0.45		3.24±0.33	
$PGF_2\alpha$	9.89±0.69**	46.52	5.54±0.42***	70.99
Control	5.75±0.31		2.23±0.58	
$PGF_2\alpha$ + Mel.	6.88±0.55	19.65	2.98±0.77	33.63
Control	6.63±0.38		2.99±0.29	
$PGF_2\alpha$ + Ind.	5.5 ±0.42	17.04	1.85±0.42*	38.13

± : Standard error
* : Significant at 5% level of probability
** : Significant at 1% level of probability
*** : Significant at 0.1% level of probability
Mel. : Melatonin
Ind. : Indomethacin

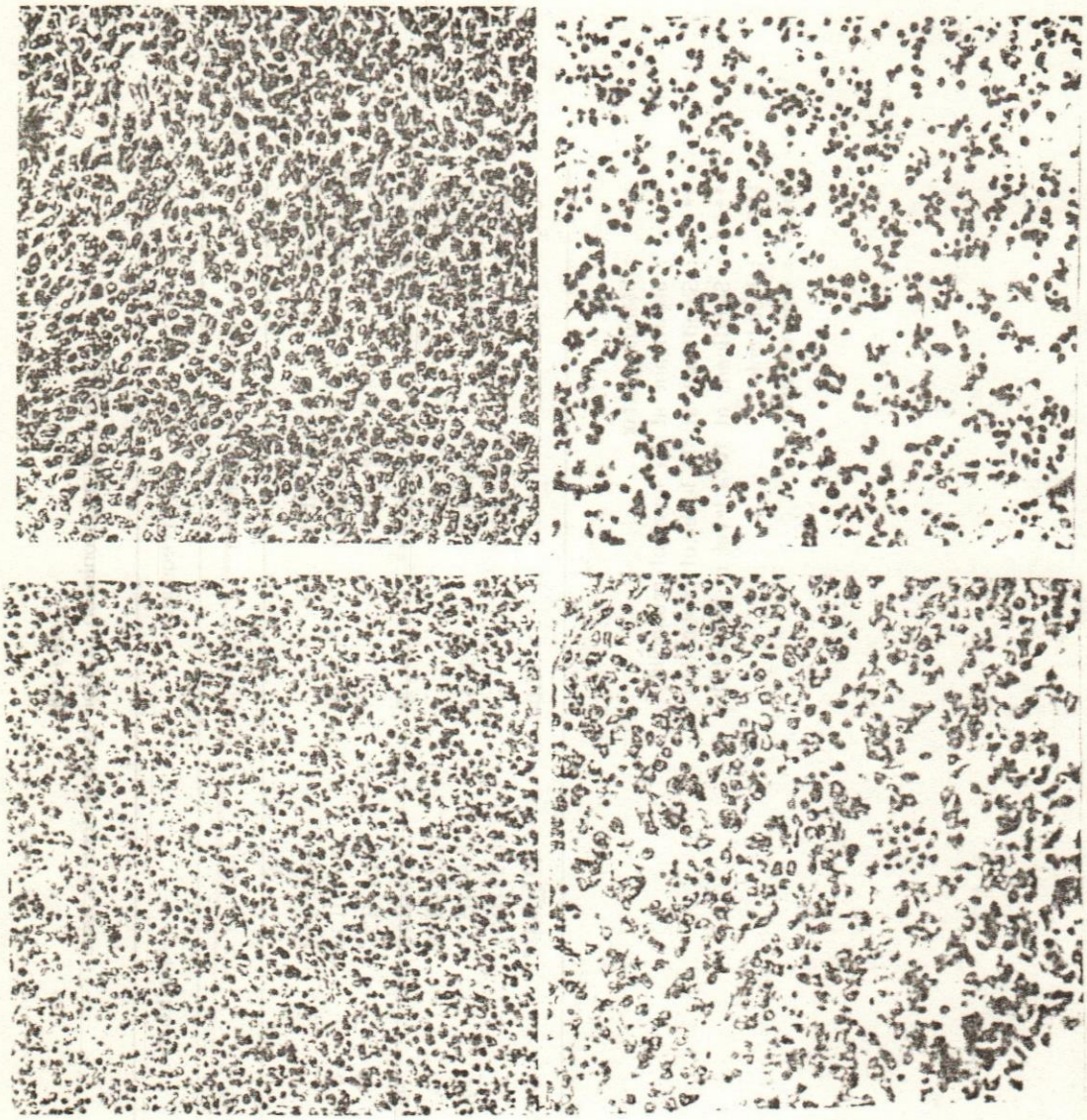

Fig. (1): Section of the embryo of mice treated with $PGF_{2\alpha}$ (B), $PGF_{2\alpha}$ + melatonin (C), $PGF_{2\alpha}$ + indomethacin (D) and control (A) (H & E X 200).

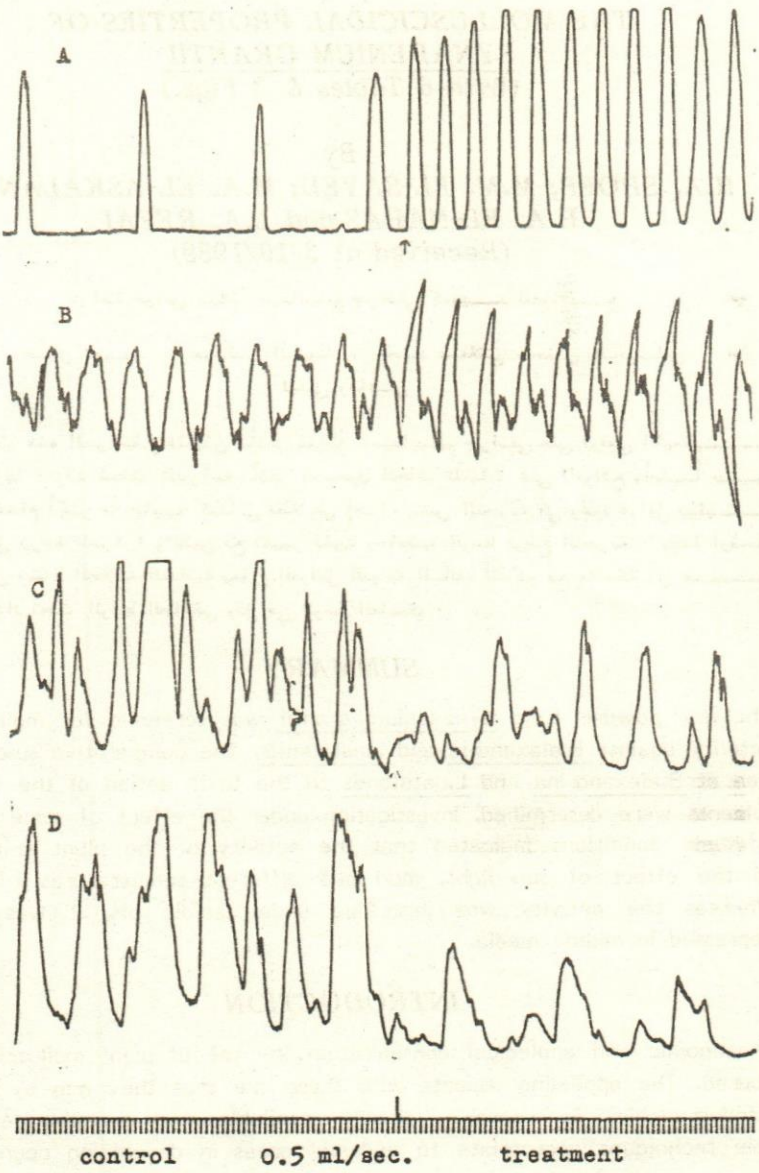

Fig. (2): Effect of $PGF_{2\alpha}$ (A), $PGF_{2\alpha}$ + melatonin (B), $PGF_{2\alpha}$ + indomethacin (C) and melatonin (D) on mice uterus.