# Inhibition of Pitocin-Induced Contractility by Melatonin (36037)

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Spontaneous and serotonin-induced contractions of the estrogenized rat uterus are inhibited by melatonin (1) as are serotonin-induced contraction of rat duodenum (2). In view of this melatonin influence on smooth muscle, the present study was designed to examine the possibility of inhibiting pitocin-induced contractions of mouse uterus and human uterus with melatonin.

*Materials and Methods.* Uterine horns from adult female mice in estrus (CF-1; 30 g) were tied at each end with silk thread and surgically removed. One end of a uterine horn was attached to a fixed glass rod within the bath containing 20 ml of de John solution (3) at 29.5° and the other end to a muscle lever recording on a revolving smoke drum. The uterine horn was allowed to equilibrate in the bath for a minimum of 10 min before each assay. Pitocin (USP, Parke Davis) was added into the bath using a 1 ml tuberculin syringe fitted with a polyethylene tube at concentration of 1.0, 2.5, 5.0, 10.0, and 20.0 mU/0.1 ml. The contraction height (mm) was measured after a 3 min exposure to the hormone and the uterus was washed three times with 50 ml of de John solution. In this manner, a pitocin standardization was obtained for the mouse uterus (Fig. 1).

The mouse uterine contraction height was related to the hormone dose; and 2.5, 5.0, and 10.0 mU of pitocin were studied (Fig. 2). Pitocin was added and the average height was recorded after 3 min. Melatonin was administered in doses of 100 to 1800 $\mu$g; and then pitocin was immediately injected in doses of 2.5 to 10.0 mU. The percentage inhibition of pitocin-induced contraction by melatonin was then calculated. A similar but more qualitative procedure was used on the human uterus. Melatonin did not change the pH of the de John solution.

A pregnant human uterus of 34 weeks was obtained from a woman after she was delivered of her child by cesarean section. Uterine strips measuring 3 cm long by 3 mm wide were cut from myometrium and used for assay in the same manner as described for mouse uterus. A pitocin dose of 100 mU produced adequate contraction. Three complete assays were performed on this patient's uterus before it became insensitive to pitocin. By using the formula $\sqrt{[\Sigma d^2/N(N-1)]}$ the standard error of the various means and applied Student's $t$ test for significant difference (4) were calculated; $p = .05$ or less was considered significant. Since only three assays were performed on the human uterus, no statistics could be utilized.

*Results.* The height of pitocin-induced contractions of mouse uterus was standarized over a 1.0 to 20.0 mU dose range (13 assays/dose group). A 10.2 $\pm$ 1.9 mm contraction was recorded for a 1.0 mU hormone injection compared to 39.5 $\pm$ 3.2 mm observed for a 20 mU dose ($p < .001$) (Fig. 1). Melatonin inhibited mouse uterine contractions produced by pitocin (Table I). The melatonin response tended to be dose-dependent; but exceptions were noted. For example, the 300 and 900 $\mu$g doses gave the same results when 2.5 mU of pitocin was injected. At the 10 mU pitocin dose, some stimulation was recorded at the 100 and 300 $\mu$g melatonin doses. Melatonin alone never produced a stimulatory response at any dose.

The human uterus contracted and relaxed at a slower rate than the mouse uterus. A significant inhibition of pitocin-induced contractions at all melatonin doses was recorded reaching a maximum 45.4% inhibition when 1800 $\mu$g was administered (Table II). Uterine

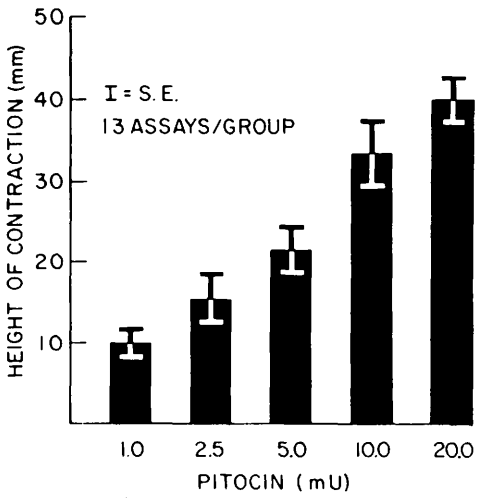


FIG. 1. Standardization of pitocin on mouse uterine contractions.

contraction required more time when melatonin was injected with pitocin than with pitocin alone.

*Discussion.* The biogenic amine, serotonin, stimulates smooth muscle. An *O*-methylation of serotonin decreased this property but *N*-acetylation caused a complete loss of the ability to contract smooth muscle (5). Rahamimoff and his associates (6) studied the relationship between melatonin and serotonin on several types of smooth muscle. Melatonin inhibited the spontaneous contractions of isolated rat duodenum at $10^{-9}$ moles/ml concentration (2) and inhibited serotonin-generated contractions in a molar ratio of 170:1. Melatonin methylation, possibly, represents a primitive method of inhibiting the biological activity of indoles to conserve oxidative mechanisms. In dogs, relatively large doses of melatonin (60 $\mu$moles/kg) cause bronchiodilation and inhibit bronchio-constriction produced by 1/60 as much serotonin. Melatonin inhibits the contraction of isolated rat uterus (1). Spontaneous uterine contractions were inhibited by 50 $\mu$g/ml melatonin but 40 times as much was required to antagonize 0.3 $\mu$g/ml of serotonin. Melatonin possesses no oxytocic action but oxytocic activity was isolated from bovine pineal gland (7). In unpublished results, we found that pineal protein-free extracts had both inhibitory and stimulatory activity on hormonal-induced (acetylcholine, serotonin, and pitocin) rat uterine contractions.

The mechanism of melatonin action on smooth muscle remains unknown but preliminary studies (1) on uterine electrical activity using external electrodes showed a decreased amplitude and frequency. The inhibitory effect might be explained by reducing muscle excitability. However, Kappers (8) could not suppress, with melatonin, the contractile response of the ileum or estrous uterus to serotonin.

In the present study, melatonin inhibited mouse and human pitocin-induced uterine contractions. Preliminary studies indicate that

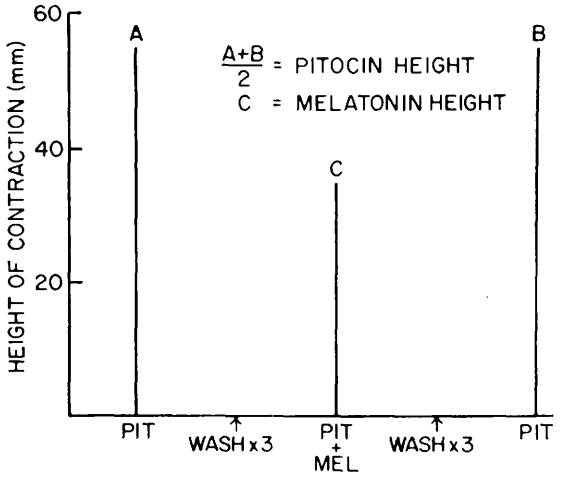


FIG. 2. A sample assay for the mouse and human uterus.

TABLE I. Inhibition of Pitocin-Induced Contraction of Mouse Uterus by Melatonin.[a]

| Melatonin dose ($\mu$g) | Inhibition of contraction (%); pitocin: (mU): 2.5 | 5.0 | 10.0 |
|---|---|---|---|
| 100 | 6.3 ± 1.7 (15) | 9.3 ± 6.9 (11) | +23.1[b] ± 9.9 (15) |
| 300 | 26.0 ± 4.7 (25) | 19.3 ± 8.8 (10) | +4.0[b] ± 4.5 (15) |
| 900 | 27.1 ± 5.7 (25) | 35.9 ± 7.0 (16) | 13.7 ± 4.9 (15) |
| 1800 | 42.2 ± 5.9 (15) | 36.7 ± 6.6 (15) | 37.6 ± 7.5 (15) |

[a] Number of assays in parentheses; ± = standard error.
[b] Exception: + = stimulation.

TABLE II. Inhibition of Pitocin-Induced (100 mU) Contraction of Human Uterus by Melatonin.

| Melatonin dose ($\mu$g) | Inhibition of contraction (%) Assays 1 | 2 | 3 | Mean |
|---|---|---|---|---|
| 100 | +10.5[b] | 5.5 | 8.6 | 1.2 |
| 300 | 5.6 | 5.6 | 50.0 | 20.4 |
| 900 | 16.1 | 48.3 | 67.3 | 43.9 |
| 1800 | 43.0 | 49.6 | 43.6 | 45.4 |

[a] Mean = average of 3 assays.
[b] + = stimulation.

melatonin produces a similar response in the *in vivo* mouse uterine preparation; however, melatonin alone does not produce a stimulatory effect.

The failure of progesterone administration to consistently prevent premature labor or saline-induced labor suggests that other hormones besides oxytocin control labor. To control premature labor, one attempts to make the uterus insensitive to oxytocin or other related substances without drugs with serious side effects. In view of the present results, one should consider the maternal and fetal pineal glands as part of the complex regulatory mechanism that constitutes uterine labor contractions. Our data suggest a competition between pitocin and melatonin but the site of action, as well as the rate of penetration, remain unknown.

*Summary.* The stimulatory effect of pitocin on estrous mouse uterus was effectively blocked by melatonin. An 1800 $\mu$g melatonin dose suppressed a 2.5 mU pitocin-induced contraction 42.4%. The inhibition of pitocin-induced (100 mU) contractions of the human uterus with the same melatonin dose was 45.4%. Our results suggest a definite antipitocin effect on the smooth muscle contractility of mouse and human uterus by melatonin.

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