# Progesterone Antagonist Mifepristone (RU 486) Decreases Cardiotoxicity of Cocaine (43536)

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*Abstract.* **RU 486, a potent progesterone antagonist, was used to determine whether RU 486 blocks the enhanced cardiotoxicity of cocaine mediated by progesterone upon rat papillary muscles. Groups of nonpregnant rats were pretreated with: progesterone for 3 days ($n$ = 12); progesterone + RU 486 for 3 days ($n$ = 12); progesterone for 3 days + single low dose RU 486, progesterone for 3 days + single high dose RU 486, and RU 486 for 3 days ($n$ = 6); or were untreated ($n$ = 12). Papillary muscles from these groups were electrically paced during exposure to cocaine concentrations ranging from $10^{-14}$ $M$ to $10^{-3}$ $M$. One group ($n$ = 6) was pretreated with RU 486, but not exposed to cocaine. The results showed that all muscles from rats pretreated with RU 486 for 3 days were functional when exposed to cocaine concentrations one to four orders of magnitude higher than tolerated by muscles from untreated or progesterone-treated rats. As indicated by alterations in contraction response patterns, RU 486 treatment for 3 days reversed progesterone-enhanced cardiotoxicity of cocaine. Single low dose and high dose RU 486 administrations also reversed progesterone's effects upon cocaine-induced cardiotoxicity.** [P.S.E.B.M. 1993, Vol 202]

The prevalence of cocaine in today's society and its impact on social, moral, and legal issues is well documented. The increased number of recent litigations, medical case reports, and clinical studies of women who have taken cocaine while pregnant illustrates the scope of consequences associated with this drug. Although most attention focuses on "cocaine babies," there is, however, evidence that pregnancy predisposes the pregnant woman to cardiovascular risks while taking cocaine. Clinical reports have associated cocaine use with placental abruption, hypertension, subarachnoid and intracerebral hemorrhages, seizures, spontaneous abortion, premature labor, and death (1–5).

Results from recent animal studies have indicated that pregnancy enhances maternal cardiovascular toxicity to cocaine, and that increased progesterone may be responsible for this enhanced toxicity. When cocaine was administered intravenously, a 2-fold greater hypertensive response occurred in pregnant than in nonpregnant sheep when given the identical dose of cocaine/kg (6). In a companion study, nonpregnant sheep were administered progesterone to produce late pregnancy levels of this steroid. The same dose of cocaine produced a 2-fold greater increase in blood pressure during progesterone treatment than it did before progesterone treatment (7). A cardiac role for this enhanced toxicity to cocaine by progesterone was suggested in that study, since the hypertensive response to exogenous norepinephrine was identical before and after progesterone treatment.

Such a role for cardiac toxicity was explored in a later study in which isolated papillary muscles from pregnant, nonpregnant, and progesterone-treated nonpregnant rat hearts were electrically paced and contraction response patterns measured. When exposed to increasing cocaine concentrations, papillary muscles from nonpregnant rats exhibited a biphasic pattern of increasing cardiac contractility (positive inotropy) followed by negative inotropy. Contractile patterns obtained from papillary muscles of pregnant and proges-

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Received December 30, 1991. [P.S.E.B.M. 1993, Vol 202]
Accepted September 21, 1992.

0037-9727/93/2023-0279$3.00/0

terone-treated nonpregnant rats did not exhibit this biphasic response. Instead, these muscles demonstrated only negative inotropy and became nonfunctional at one to three orders of magnitude *lower* cocaine concentrations than muscles from untreated nonpregnant rats (8).

From the above animal studies, progesterone appears to increase the toxicity of cocaine. Whether this increased toxicity can be blocked by a progesterone antagonist is the focus of the present study. The novel progesterone receptor antagonist mifepristone, or RU 486, is recognized as an abortifacient (9–11) because of its great affinity for and inactivation of the progesterone receptor. In addition to being a potent progesterone antagonist, RU 486 has affinity for the glucocorticoid receptor (12–15) and has also been shown to release prostaglandins from human myometrial cultures (16). RU 486 also interacts weakly with androgen receptors (17) and has been observed to block effects of testosterone in castrated male rats (12). In contrast, RU 486 is reported to have no antiestrogenic nor antimineralocorticosteroidal effects (12).

The overall purpose of the present study was to determine whether RU 486 reverses progesterone-enhanced cardiotoxicity of cocaine after repeated administration of progesterone in nonpregnant rats. This exposure protocol typifies the clinical situation in which acute cocaine exposure can occur during a sustained state of elevated systemic progesterone, such as pregnancy. Other specific questions addressed in this study were: does blockade by RU 486 require daily administration or can it be accomplished with only single low or high dose administration?; does RU 486 alone, in the absence of exogenous progesterone, affect cocaine-induced cardiotoxicity?; and, does RU 486 influence cardiac contractility in the absence of cocaine?

## Methods

This study was divided into four separate phases conducted on cardiac papillary muscles obtained from nonpregnant female Long-Evans rats weighing 250–300 g. The first phase was designed to determine whether RU 486, when coadministered *daily* with progesterone for 3 days, would block progesterone's enhancement of cocaine-induced cardiotoxicity. To do so, papillary muscle responses to cocaine from hearts obtained from untreated rats ($n = 12$), rats treated for 3 consecutive days with progesterone, 7 mg/kg im ($n = 12$), and rats treated for 3 consecutive days with progesterone, 7 mg/kg im, plus RU 486, 10 mg/kg oral administration ($n = 12$) were examined. The dose of progesterone given to nonpregnant rats produced plasma progesterone levels comparable to those obtained from late pregnancy (8). The 3-day time course of progesterone administration duplicated the time course previously utilized in other studies (6, 18).

The second phase was designed to determine whether a *single* dose of RU 486 given just before decapitation could accomplish the same blocking action as when given for 3 days. Papillary muscle responses to cocaine were studied in hearts obtained from two groups of rats, both of which had been treated for 3 days with progesterone, 7 mg/kg im. One hour before decapitation, each group was treated with either a low dose, 10 mg/kg orally ($n = 6$), or a high dose, 50 mg/kg orally ($n = 5$), of RU 486. These two doses were selected to determine whether the results of this treatment were dose dependent.

The third phase was designed to determine whether RU 486, in the absence of exogenous progesterone, influences cocaine-induced cardiotoxicity. For this, papillary muscle responses to cocaine from hearts obtained from rats treated for 3 days with RU 486 alone ($n = 6$) were studied.

The final phase was to determine whether RU 486 alone affected papillary muscle contractility. In the absence of cocaine, papillary muscle responses were recorded during 7 hr of stimulation from hearts obtained from rats that had been treated with RU 486 for 3 days ($n = 6$).

All studies conformed to the principles of animal care as indicated by the American Physiological Society, the Animal Welfare Act of 1970, and the National Institutes of Health Policy on Laboratory Animals.

Progesterone (Lilly, Indianapolis, IN), 7 mg/kg, was injected intramuscularly into a rat hind limb for 3 consecutive days. Mifepristone, RU 486, was supplied as a gift from Roussel Uclaf, Romainville, France. RU 486 was prepared for oral dosing by dissolving the dose in a solution of 0.25% carboxymethyl cellulose, 0.20% polysorbate 80, and 99.55% distilled water. Each oral dose of RU 486 was administered to the rat via syringe through an 18 gauge × 3 in, 2.25-mm diameter ball end, curved animal feeding needle (Harvard Apparatus, South Natick, MA). For animals coadministered progesterone and RU 486 for 3 days, RU 486 was given within minutes after the progesterone injection. In all cases, regardless of treatment profile, decapitation occurred 1 hr after the final administration of either progesterone or RU 486.

In this study, the isolated muscle bath preparation was used as previously described (8, based on [19]). After decapitation, a blood sample from each rat was collected and later analyzed for plasma progesterone by $^{125}$I radioimmunoassay (Diagnostic Products Corp., Los Angeles, CA). Posterior papillary muscles were then dissected from the left ventricle and immediately immersed in bicarbonate Krebs solution bubbled with 95% $O_2$ and 5% $CO_2$ (pH 7.4) and a calcium concentration of 1.1 m*M*. The bath chamber was filled with bicarbonate Krebs solution and a circulating water jacket maintained bath temperature at 28°C. One end

of the muscle was attached to a clip attached to an isometric force transducer (FT03; Grass, Quincy, MA) and the other end was attached to a clip in a fixed position at the bottom of the bath chamber. Muscle contractions were recorded on a Gould Brush 440 recorder (Gould Corp., Cleveland, OH). Stimuli, 0.3 Hz, 3 msec duration, and voltage 25% above threshold, were applied through platinum electrodes in the muscle bath.

All papillary muscles were allowed to contract for 60 min before addition of cocaine to the bath, and then were studied for periods not exceeding 7 hr of stimulation. After this 60-min equilibration period, each muscle was gradually stretched until the force of contraction was maximum ($L_{max}$) and this length was measured for later calculation of muscle cross-sectional area. From each muscle contraction, the parameters of peak tension ([PT] maximum height of the curve), time to peak tension ([TPT] time from initiation of muscle contraction to PT), half relaxation time ([½RT] time after PT to ½ PT), and the rate of tension formation ([dT/dt] slope of the contraction curve) were measured. Contraction curves were obtained at 1, 2, 5, 10, 15, 20, 25, and 30 min after each addition of cocaine HCl (Mallinckrodt, Inc., St. Louis, MO). Only the maximum increase or decrease in dT/dt and its corresponding parameter measurements from each muscle were used for subsequent data analysis. This change typically occurred at approximately 10 min after addition of each new cocaine concentration to the bath. If no changes in dT/dt measurements occurred during the 30-min observation period, measurements made 10 min after addition of cocaine were used. Cocaine was added at 30-min intervals to the muscle bath to produce final molar concentrations of $10^{-14}$ *M*, $10^{-12}$ *M*, $10^{-10}$ *M*, $10^{-9}$ *M*, $10^{-8}$ *M*, $10^{-7}$ *M*, $10^{-6}$ *M*, $10^{-5}$ *M*, $10^{-4}$ *M*, and $10^{-3}$ *M*. These concentrations were selected on the basis of our previous study (8). Since a no-effect concentration was desired, $10^{-14}$ *M* was selected as the lowest initial cocaine concentration. The highest concentration used was $10^{-3}$ *M*, because at this concentration all muscles were nonfunctional.

A muscle was determined to be nonfunctional in any experiment if it failed to respond to stimulation within 10 min after addition of a new cocaine concentration. In all cases involving nonfunctional muscles, the muscle remained nonfunctional throughout the 30-min interval despite continued stimulation. The response measurement for that muscle at that concentration was entered as zero.

At the conclusion of each experiment, the papillary muscle was weighed. Final calculations of dT/dt and PT were standardized for muscle mass by dividing each measurement by the cross-sectional area (Cross-sectional area = weight of muscle/$L_{max}$ × 1.05 g/mm$^3$ [density of cardiac muscle]).

Statistical analysis of dT/dt, PT, TPT, and ½ RT consisted of a two-way (factors = concentration and treatment) repeated-measures analysis of variance. A post-hoc least significant difference range test was used to determine differences among means when a significant analysis of variance was obtained. All data satisfied tests of normal distribution and variance equality. Significant differences between mean plasma progesterone levels were determined by one-way analysis of variance and post-hoc least significant difference test. Plasma progesterone data demonstrated normality but not equal variance. Cube root transformation of these data yielded equal variance yet did not produce any alteration in significance testing when compared with results from the original data analysis. Significance was set at $P < 0.05$.

## Results

All groups receiving progesterone treatment had significantly higher plasma progesterone levels than those groups not receiving progesterone treatment (Table I). Of those receiving progesterone treatment, the highest mean progesterone level was observed in the group receiving progesterone + RU 486 for 3 days. This level was statistically different from the group treated with progesterone only for 3 days and the group treated with progesterone + single low dose (10 mg/kg) RU 486 1 hr before decapitation, but not from the group treated with progesterone + single high dose (50 mg/kg) RU 486 1 hr before decapitation. The groups not receiving progesterone had the lowest progesterone levels of all groups.

**Table I.** Plasma Progesterone Levels[a]

| Treatments | Progesterone level (ng/ml) |
|---|---|
| Untreated | 17.5 ± 1.6 |
| Progesterone 7 mg/kg im for 3 days | 97.3 ± 7.9[b] |
| Progesterone 7 mg/kg im + RU 486 10 mg/kg orally for 3 days | 150.2 ± 11.4[b–d] |
| Progesterone 7 mg/kg im for 3 days + RU 486 10 mg/kg orally 1 hr before decapitation | 83.6 ± 4.2[b] |
| Progesterone 7 mg/kg im for 3 days + RU 486 50 mg/kg orally 1 hr before decapitation | 130.2 ± 10.2[b,c] |
| RU 486 10 mg/kg orally only for 3 days (cocaine) | 26.5 ± 1.9 |
| RU 486 10 mg/kg orally only for 3 days (control) | 26.0 ± 3.1 |

[a] Values represent mean ± SE.
[b] Significant difference from nonprogesterone-treated groups.
[c] Significant difference from progesterone for 3 days + 10 mg/kg of RU 486 on day of decapitation group.
[d] Significant difference from progesterone-treated group and from progesterone for 3 days + 10 mg/kg of RU 486 on day of decapitation group.

**Baseline Contractility Measurements Prior to Cocaine Exposure.** The groups in the first phase of the study (untreated, progesterone only for 3 days, and progesterone + RU 486 for 3 days) each had significantly different baseline dT/dt values (Fig. 1, first panel) prior to cocaine exposure. Since dT/dt values were derived from PT and TPT, differences in PT and/or TPT values may contribute to differences in dT/dt. Baseline PT values (Fig. 1, second panel) of the progesterone-treated group and progesterone + RU 486 for 3 days-treated groups were different from each other. However, baseline PT of the untreated group was not significantly different from either of the other two groups. Baseline TPT values (Fig. 1, third panel) were not significantly different from each other. However, a shorter TPT, exhibited by the progesterone only for 3 days-treated group, as well as the increased PT, contributed to the increased baseline dT/dt demonstrated by progesterone treatment. In this three-group comparison, the differences in baseline dT/dt are primarily a reflection of differences in PT. Both progesterone-treated groups had significantly shorter baseline ½ RT values (Fig. 1, fourth panel) than the untreated group.

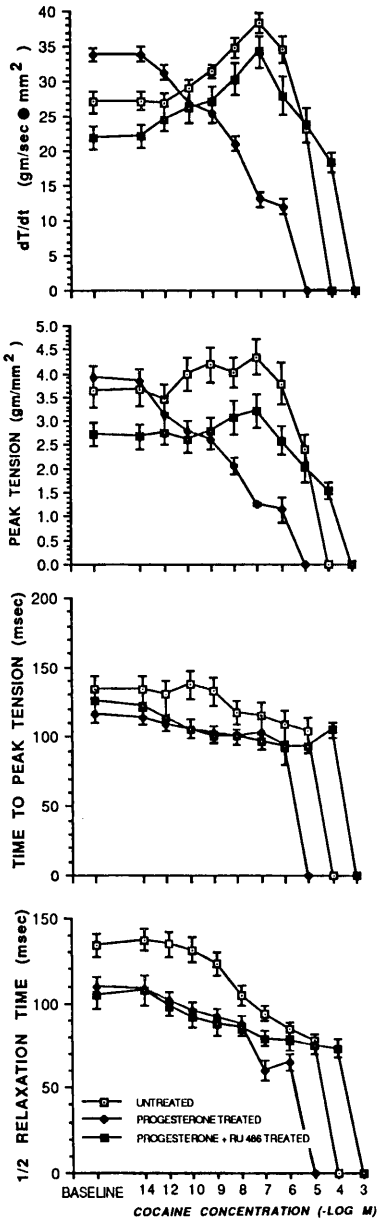


**Figure 1.** Mean ± 1 SE of parameters measured from isolated papillary muscles obtained from untreated nonpregnant rats ($n$ = 12), rats pretreated with progesterone, 7 mg/kg for 3 days ($n$ = 12), and rats pretreated with progesterone, 7 mg/kg + RU 486 for 3 days ($n$ = 12), in response to increasing cocaine concentrations.

**Muscle Function During Cocaine Exposure.** All papillary muscles from all groups were functional during baseline measurements and when exposed to cocaine administrations ranging from $10^{-14}$ $M$ to $10^{-8}$ $M$ (Table II). The progesterone-only-treated group was more sensitive to lower concentrations of cocaine when compared with all other groups. Beginning with the $10^{-7}$ $M$ concentration, the number of nonfunctional papillary muscles from the progesterone-only-treated group increased until $10^{-5}$ $M$, the concentration at which all muscles in this group were nonfunctional. All papillary muscles from the untreated group were functional throughout all cocaine concentrations ranging from $10^{-14}$ $M$ to $10^{-5}$ $M$, but all became nonfunctional at $10^{-4}$ $M$. Papillary muscles from *all* RU 486-treated groups were functional until $10^{-3}$ $M$ cocaine concentration, the highest cocaine concentration used in this study.

**Characteristics of Muscle Contractility During Cocaine Exposure.** A plot of maximum dT/dt response values with increasing cocaine concentrations of the untreated, progesterone only for 3 days-treated, and progesterone + RU 486 for 3 days-treated groups shows differing patterns of response (Fig. 1, first panel). In both the untreated and the progesterone + RU 486 for 3 days-treated groups, dT/dt values increased (positive inotropy) with increasing cocaine concentration, reaching a peak by $10^{-7}$ $M$ concentration. Thereafter, dT/dt values decreased (negative inotropy) until muscles were nonfunctional by $10^{-4}$ $M$ or $10^{-3}$ $M$. This pattern of positive inotropy followed by negative inotropy was not evident in dT/dt results obtained from the progesterone only for 3 days-treated group. Beginning with the $10^{-12}$ $M$ concentration, dT/dt values from the progesterone only for 3 days-treated group demonstrated negative inotropy with increasing cocaine concentrations until all muscles were nonfunctional by the $10^{-5}$ $M$ concentration. The positive inotropy observed in the untreated group appears to be a result of an increase in PT with increasing cocaine concentration while there is little or no change in TPT. Positive inotropy observed in the progesterone + RU 486 for 3 days-treated group appears to be a result of decrease in TPT from $10^{-14}$ $M$ to $10^{-10}$ $M$, and a result of a combined increase in PT and decrease in TPT from

**Table II.** Number of Functional Papillary Muscles

| | .Cocaine concentration (Log M) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Control | −14→−9 | −8 | −7 | −6 | −5 | −4 | −3 |
| Nonpregnant | 12/12[a] | 12/12 | 12/12 | 12/12 | 12/12 | 12/12 | 0/12 | 0/12 |
| Nonpregnant progesterone treated | 12/12 | 12/12 | 12/12 | 3/12 | 2/12 | 0/12 | 0/12 | 0/12 |
| Nonpregnant progesterone RU 486 treated | 12/12 | 12/12 | 12/12 | 12/12 | 12/12 | 12/12 | 12/12 | 0/12 |

[a] *n/n* = Number of functional muscles/total number of muscles tested.

$10^{-9}$ *M* to $10^{-7}$ *M*. Indeed, the differences in dT/dt between the untreated and the progesterone + RU 486 for 3 days-treated groups parallel each other with increasing cocaine concentrations. The negative inotropy observed in all groups appears to be a result of decreases in PT with increasing cocaine concentrations. The ½ RT values decrease with increasing cocaine concentration in all groups, with both progesterone for 3 days-treated groups having a consistently shorter ½ RT until the $10^{-6}$ *M* cocaine concentration.

**Effect of RU 486 Single Dose Exposure.** Two groups of rats were treated with progesterone for 3 days and then received either 10 mg/kg (low dose) or 50 mg/kg (high dose) of RU 486 1 hr before decapitation (Fig. 2). Baseline values of dT/dt, PT, and ½ RT of these two groups were not significantly different from each other. Baseline TPT values were significantly different from each other and the lower baseline TPT of the low dose RU 486-treated group probably accounts for the slightly elevated baseline dT/dt of the same group.

With increasing cocaine concentrations, the patterns of muscle response were different between the groups receiving either high or low single dose RU 486. The higher single dose of RU 486 (50 mg/kg) produced the biphasic dT/dt response typical of the untreated and the progesterone + RU 486 for 3 days-treated groups, with the peak occurring at $10^{-8}$ *M* cocaine concentration. In sharp contrast, the biphasic response was conspicuously absent in the group receiving the lower single dose of RU 486 (10 mg/kg). Like the responses of the progesterone only for 3 days group, this group receiving the single lower dose of RU 486 demonstrated only negative inotropy with increasing cocaine concentrations. The positive inotropy of the RU 486 single high dose group was associated solely with increases in PT with increasing cocaine concentrations, since no significant differences in TPT occurred with the exception of the baseline values as mentioned above. As with muscles from the group treated with progesterone + RU 486 for 3 days, all papillary muscles in these two groups given single dose RU 486 were functional until $10^{-3}$ *M* cocaine concentration (Table II). Although decreasing with increasing cocaine con-

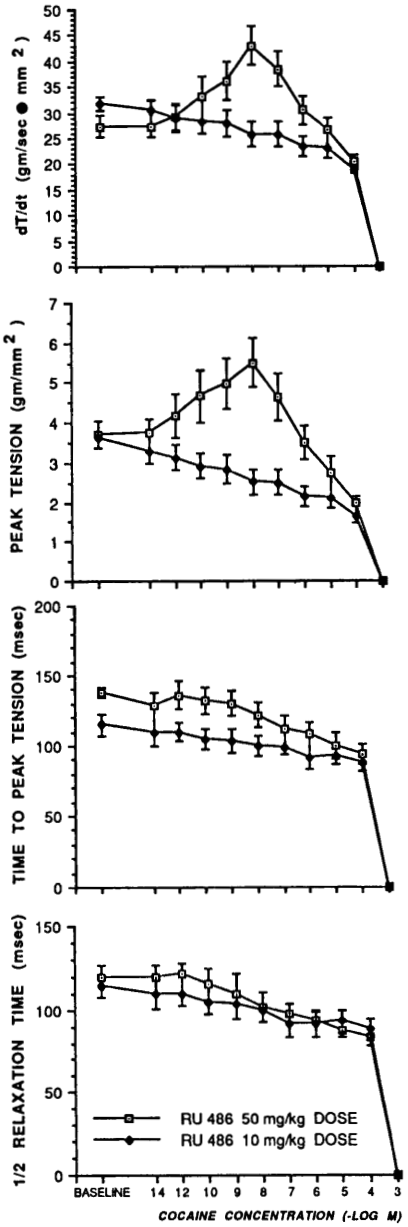


**Figure 2.** Mean ± 1 SE of parameters measured from isolated papillary muscles obtained from rats pretreated with progesterone, 7 mg/kg for 3 days + RU 486 at 10 mg/kg for 1 hr before decapitation ($n$ = 6), and from rats pretreated with progesterone, 7 mg/kg for 3 days + RU 486 at 50 mg/kg for 1 hr before decapitation ($n$ = 5), in response to increasing cocaine concentrations.

centrations, ½ RT values were not different between the low and high dose RU 486 groups.

**Effect of RU 486 Treatment for 3 Days.** One group of rats was treated with RU 486 only, 10 mg/kg, for 3 days before decapitation (Fig. 3). Papillary muscle responses from this group demonstrated baseline values of dT/dt, PT, and TPT that were in between values of the untreated and the progesterone + RU 486 for 3 days-treated groups. Baseline ½ RT values were similar to those of progesterone-treated groups. With increasing cocaine concentrations, papillary muscles from this group demonstrated a biphasic response of positive then negative inotropy, with the peak occurring at $10^{-10}$ *M* cocaine concentration. As with other groups receiving RU 486 treatment, all muscles were functional until $10^{-3}$ *M* cocaine concentration. As shown in Figure 3, changes in inotropy in this group occurred as a result of changes in PT.

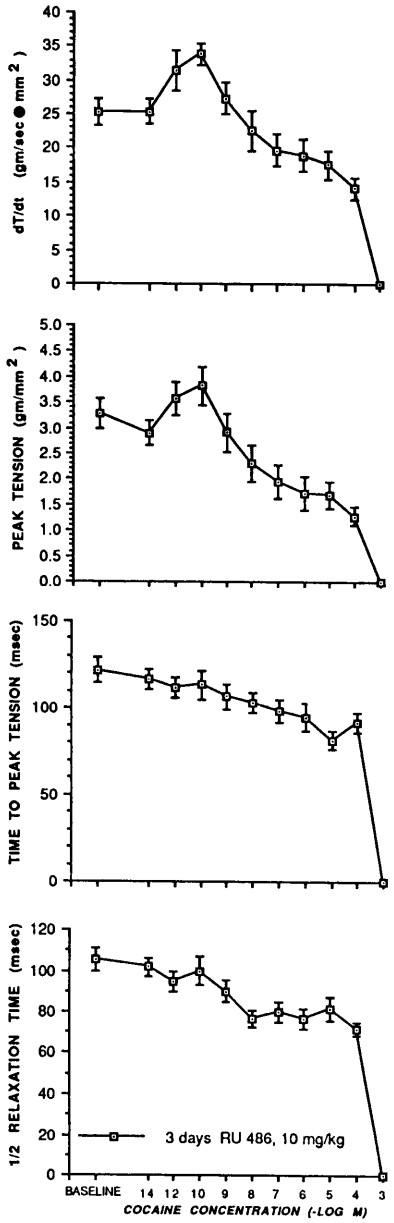


**Figure 3.** Mean ± 1 SE of parameters measured from isolated papillary muscles obtained from rats pretreated with RU 486, 10 mg/kg for 3 days, in response to increasing cocaine concentrations.

**Effect of RU 486 Treatment upon Cardiac Contractility in the Absence of Cocaine.** One group of rats was treated with RU 486, 10 mg/kg, for 3 days before decapitation, and papillary muscles obtained from this group were stimulated for 7 hr in the absence of cocaine. The lack of change in any of the response values demonstrated that RU 486 alone had no effect upon papillary muscle contractility within the time frame in which all data in this study were collected.

## Discussion

In a prior study, we demonstrated that isolated papillary muscles obtained from nonpregnant rats exhibit a biphasic contractile pattern when stimulated during exposure to increasing concentrations of cocaine (8). In contrast, papillary muscles obtained from pregnant or progesterone-treated nonpregnant rats exhibit only negative inotropy and become nonfunctional at lower concentrations of cocaine than papillary muscles obtained from nonpregnant rats (8). Data obtained from untreated and progesterone only treatment groups in the current study are consistent with these previous findings of producing negative inotropy and nonfunctionality following a dose-related exposure to cocaine. Since progesterone receptor occupancy results in a cascade of transcriptional events, 3 days of systemic progesterone therapy was initiated to produce comparable plasma levels of progesterone noted during late gestation in the rat. The progesterone antagonist RU 486 was administered to rats to block progesterone receptor interaction in order to determine whether enhanced cocaine cardiotoxicity was mediated through the progesterone receptor. To our knowledge, this report is the first in which the effects of RU 486 on cardiac muscle have been studied. The results presented in this study indicate that RU 486 pretreatment reversed or eliminated progesterone's enhancement of cocaine-induced cardiotoxicity.

Progesterone is essential for implantation and maintenance of pregnancy. Blood progesterone levels increase during the menstrual cycle and with progression of pregnancy once implantation occurs. In addition to being found in reproductive organs and the brain, cytosolic progesterone receptors have been identified in cardiac muscle (20). Binding of endogenous or exogenous progesterone with these receptors produces a complex which then enters the nucleus, interacts with nuclear chromatin, and results in a transcriptional cascade. RU 486 is a potent antiprogesterone agent that interferes with the binding of progesterone with the cytosolic receptor and, when given in early pregnancy, prevents implantation by inducing luteolysis. RU 486

in the current study altered cardiotoxicity of cocaine associated with progesterone administration. Administration of RU 486 along with progesterone for 3 days restored the cocaine-induced biphasic response pattern of positive and negative inotropy typical of muscles from untreated rats.

In this study, the absence of a biphasic response pattern to cocaine correlated with cardiotoxicity. The rats treated with progesterone only for 3 days demonstrated only negative inotropy with increasing cocaine concentrations. Subsequently, all papillary muscles in this group were nonfunctional at lower cocaine concentrations than either the untreated group or the progesterone + RU 486 for 3 days-treated group. In contrast, all papillary muscles from rats treated with RU 486 retained the ability to function when exposed to increasing cocaine concentrations and only became nonfunctional at $10^{-3}$ *M* cocaine concentration. This biphasic response pattern was observed even for those rats that did not receive any exogenous progesterone but received only RU 486. The only group treated with both progesterone for 3 days and RU 486 that deviated from the biphasic response pattern was the group treated with single low dose RU 486 (10 mg/kg). This characteristic may be due to small amounts of RU 486 competing with high levels of progesterone for available progesterone receptor sites.

The observation that plasma progesterone levels were highest in the group receiving progesterone + RU 486 for 3 days indicates that RU 486 is preventing binding of exogenous progesterone to the progesterone receptor. That progesterone levels in this group differed significantly from that of the progesterone- only-treated group suggests that RU 486 is probably displacing endogenous progesterone as well. Plasma progesterone levels alone certainly are not indicative of progesterone receptor activation or inactivation after RU 486 administration. However, progesterone levels have been reported to increase after RU 486 administration and subsequent to luteolysis (12, 21). Although RU 486 has approximately five times the affinity for the progesterone receptor as the natural agonist (21), affinity for receptor does not infer efficacy (21). This has been demonstrated for a number of steroid antagonists and is associated with half-life of the antagonist, continuous and/or repeated antagonist administration, and stabilization of the cytosolic receptor-antagonist complex (9). RU 486 has a relatively long half-life of 20 hr in humans (22).

The papillary response patterns of the two groups treated with single low and high dose administrations of RU 486 1 hr before decapitation indicate that RU 486 acts rapidly and that its effect upon cocaine cardiotoxicity is dose dependent. Differences in progesterone levels between single low and high dose RU 486-treated groups suggest that high dose RU 486 administration displaces more progesterone than low dose treatment. Although rats treated with low dose RU 486 did not demonstrate a biphasic response, all muscles remained functional until $10^{-3}$ *M* cocaine concentration. This indicates that even low dose RU 486, given as a single administration, partially prevented the increased cocaine cardiotoxicity mediated by progesterone. Muscles from the group treated with single high dose RU 486 were also functional until the $10^{-3}$ *M* cocaine concentration, but, in addition, exhibited the biphasic contractility pattern typical of papillary muscles of untreated rats. Since these effects of single low or high dose RU 486 occurred following administration 1 hr before decapitation, the progesterone-enhanced cardiotoxic effects of cocaine were rapidly reversed. This rapid reversal suggests that progesterone's effects upon cocaine cardiotoxicity are associated with modification of proteins or altered regulation of sites not related to transcriptional effects of progesterone. Since alterations in intracellular $Ca^{2+}$ produce changes in inotropy (23), progesterone's enhancement of cocaine cardiotoxicity may be localized in the regulation of intracellular $Ca^{2+}$ via $Ca^{2+}$ channels or modulation of intracellular $Ca^{2+}$ release by the sarcoplasmic reticulum.

The presence of progesterone receptors in cardiac muscle (20) suggests that progesterone may play a role in physiologic adaptation of the adult heart during pregnancy. During pregnancy, there is increased blood volume and cardiac output. An increased sympathetic tone is thought to compensate for this increased cardiac load (24). Indeed, enhanced sympathetic tone via increased catecholamine release would increase cardiac contractility. Alternatively, progesterone receptor activation may be a triggering mechanism for increased cardiac contractility during pregnancy. Previous investigators (25, 26), examining progesterone actions upon papillary muscles, have indicated that progesterone is cardiodepressive, that is, progesterone produces decreased cardiac contractility. In these studies, however, progesterone was added directly to bath solutions and may not reflect the *in vivo* situation. Moreover, *in vivo* interactions of progesterone at other foci may contribute to associated or additive effects of endogenous or exogenous progesterone. In contrast to observations by others, progesterone administration for 3 days in the current study and pregnancy from a previous study (8) produced *increases* in baseline cardiac contractility.

It was expected that papillary muscles from rats administered RU 486 for only 3 days in the absence of exogenous progesterone administration would produce responses to increasing cocaine concentrations typical of muscles obtained from untreated rats. Although a biphasic contractility response occurred, peak dT/dt was noted at $10^{-10}$ *M*, three orders of magnitude lower concentration of cocaine than the concentration at which peak dT/dt from the untreated group occurred.

Based upon the rapid decline in dT/dt values following the peak dT/dt in other groups, we would have expected that dT/dt values and muscle functionality would likewise have deteriorated rapidly. However, muscles from this group remained functional until the cocaine concentration of $10^{-3}$ *M*, which was typical of muscles from other rats treated with RU 486. This protracted period of negative inotropy from peak dT/dt until muscles became nonfunctional suggests that RU 486 protects the papillary muscle from the toxic effects of cocaine.

RU 486 is also a glucocorticoid receptor antagonist and competes effectively with the glucocorticoid receptor agonist dexamethasone for binding with the glucocorticoid receptor (13). The protective effects of RU 486 against progesterone's enhancement of cocaine cardiotoxicity in the current study could conceivably be due to interaction at the glucocorticoid receptor as well as the progesterone receptor. However, progesterone itself is a glucocorticoid receptor antagonist (27) and the high progesterone levels in all groups treated with progesterone would bind to available glucocorticoid receptors and block RU 486 action at the glucocorticoid receptor. Because of this dual progesterone-RU 486 antagonism at the glucocorticoid receptor, the difference of the biphasic contractility response pattern to cocaine exhibited by the progesterone + RU 486-treated group and the negative inotropy response pattern exhibited by the progesterone only-treated group effectively rules out a possible role for the glucocorticoid receptor in progesterone-mediated cardiotoxicity of cocaine.

The results of this study indicate that RU 486, a progesterone antagonist, reverses progesterone-enhanced cardiotoxicity of cocaine upon rat papillary muscles. The mechanism(s) for the protective actions of RU 486 against cocaine cardiotoxicity remains to be explored in greater detail. In the design of the current study, RU 486 was given as pretreatment before cardiac muscle exposure to cocaine in the muscle bath in order to counter the effects of exogenous progesterone treatment. Since in this study and in previous studies exogenous progesterone administration has been shown to increase toxicity to cocaine, it is possible that cocaine-mediated cardiotoxicity *in vivo* is related to steroid hormonal interaction. If so, administration of RU 486 may be effective in reducing the cardiotoxic effects of cocaine during cases of cocaine overdose. Since RU 486 has greater affinity for the progesterone receptor than the natural agonist, RU 486 could effectively displace the agonist and inactivate steroid-mediated cocaine cardiotoxicity.

This work was supported by National Institutes of Health, National Institute on Drug Abuse (NIDA) Grants DA04415 and DA07232. Dr. A. Sharma was the recipient of a postdoctoral fellowship from NIDA Training Grant DA07232.

This work was presented in part at the 38th Annual Meeting of the Society for Gynecologic Investigation, San Antonio, Texas, March 20–23, 1991, Abstract No. 224.

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