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Ketamine as an Anesthetic for Obtaining Plasma for Rat Prolactin Assays (40273)

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Various procedures have been utilized to obtain blood from laboratory rats for assay of plasma prolactin levels. Because of the effect of stress and general anesthetic agents on plasma prolactin levels (1–4), blood sampling procedures which do not themselves affect plasma prolactin levels are limited. Dohler *et al.* (5) recently compared the influence of four methods of blood collection under three anesthetics, ether, chloroform and pentobarbital, to decapitation on the release of prolactin; in all instances they observed an increase in plasma prolactin ranging from 2- to 13-fold.

Lawson and Gala (4) reported that ketamine, which is a dissociative anesthetic, not a general anesthetic (6), produced no effect on plasma prolactin levels at 10, 30, 60 and 120 min after intraperitoneal (ip) or intra-arterial (i.a.) injection in ovariectomized rats with indwelling catheters. Lawson and Gala (7) subsequently reported that ketamine, 100 mg/kg, ip, also produced no change in plasma prolactin levels in catheterized, ovariectomized, estrogen-treated Sprague-Dawley rats, with sampling at 10, 30, 60 and 120 min after injection. However, ketamine, 50 mg/kg, i.a., significantly decreased plasma prolactin levels at 10, 60 and 120 min. They suggested that ketamine differed from other anesthetics in its effects on prolactin secretion because it induced only stage II anesthesia.

We were interested in determining what effects if any, ketamine had on plasma prolactin levels in male rats. Since ketamine has been shown to inhibit both dopamine and serotonin uptake (8, 9), two neurotransmitters which have a profound effect on rat prolactin secretion (10, 11), it was of further interest to see if ketamine affected baseline prolactin levels or the reserpine, α-methylparatyrosine (AMPT)- or 5-hydroxytryptophan (5-HTP)-induced increase in prolactin secretion. Drugs which inhibit 5-HT uptake will promote the increase in prolactin produced by 5-HTP (12).

Methods. Male Sprague-Dawley rats (Sprague-Dawley, Inc., Madison, WI) weighing 200–225 g were housed for at least 1 week in a temperature-controlled (25°) and light-controlled (6 AM–8 PM light period) animal room. They received food and water *ad libitum*. Ten groups of five rats each had catheters placed in the right jugular vein under pentobarbital anesthesia (13). The catheters were kept patent with flushing with saline on alternate days. These rats were handled frequently and accustomed to the procedure of withdrawal of 0.3 ml blood. These rats received no anesthesia at the time of blood withdrawal. Another ten groups of five rats each were administered ketamine, 100 mg/kg, ip Immediately after they became unresponsive to toe pinch (usually 2–3 min) blood was withdrawn from the inferior vena cava. Finally, ten groups of five rats were rapidly decapitated.

Reserpine, 5 mg/kg, ip, was given 3 hr 55 min or 3 hr 30 min before ketamine, 100 mg/kg, ip, or saline. Rats were sacrificed at 4 hr after reserpine. AMPT, 100 mg/kg, ip, was given 15 min before ketamine, 100 mg/kg, ip and rats were sacrificed 15 min later. To determine the effects of ketamine on 5-HTP-induced increases in plasma prolactin, ketamine, 25, 50, and 100 mg/kg, ip, were given 30 min before 5-HTP, 30 mg/kg, ip. For comparison purposes, one group of rats was pretreated with fluoxetine (Lilly 110140), a known 5-HT reuptake blocker (14), another was pretreated with saline, followed by 5-HTP, as described for the ketamine-pretreated rats.

Following sacrifice, plasma samples were frozen and assayed later for prolactin by a modification of a double antibody radioimmunoassay originally developed for human prolactin assay (15). Prolactin levels are ex-

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pressed in terms of NIAMDD-rat prolactin-RP-1. All samples utilized in this report were assayed together. The sensitivity of the assay is 1.0 ng/ml. The intra-assay variation is less than 5%.

To determine if there was a difference in basal prolactin levels between types of sacrifice, the means for the 10 groups of each type were compared with a one way analysis of variance (ANOVA). To examine for differences in variance within each of the three treatments, a completely randomized hierarchical analysis of variance was performed (16). The effect of drugs on the increase in prolactin produced by 5-HTP was determined by an ANOVA.

Ketamine HCl was generously supplied by Parke-Davis-Warner-Chilcott, Inc., Ann Arbor, Mich. Alpha-methylparatyrosine methylester and 5-hydroxytryptophan methylester were purchased from Sigma, Inc., St. Louis, MO. Reserpine was obtained from Ciba-Geigy Corp., Summit, NJ. Fluoxetine was a gift of Eli Lilly, Co., Indianapolis, IN. All drug doses refer to the salt form.

Results. Prolactin levels for the various types of sacrifice are summarized in Table I. The median, range and coefficient of variation (c.v.) were calculated utilizing the mean data for each group of five rats.

The results of an ANOVA indicated there was no significant difference between any of the three methods of blood collection. However, five of the ten groups of catheter samples had mean levels that exceeded the highest mean of the ketamine groups (10.2 ng/ml). Only one of the decapitated groups had a mean plasma prolactin which exceeded 10.2 ng/ml. The ketamine-treated group had the lowest prolactin levels and the smallest coefficient of variation of the three types of treatment.

Ketamine did not significantly affect the increase in plasma prolactin levels produced by reserpine or AMPT (Table II).

5-Hydroxytryptophan, 30 mg/kg, or fluoxetine, 10 mg/kg, did not increase plasma prolactin levels (Table III). Fluoxetine, together with this dose of 5-HTP, produced a very significant increase in plasma prolactin. However, none of the three doses of ketamine, plus 5-HTP had any effect on plasma prolactin levels. Fluoxetine plus ketamine, 100 mg/kg, also did not augment plasma prolactin.

Discussion. The results of the studies in untreated male rats strongly indicate that anesthesia with ketamine does not affect plasma prolactin levels. Blood obtained from the inferior vena cava within 3 min of administration of ketamine has levels of prolactin not significantly different from that obtained from decapitated rats or from rats with indwelling venous catheters. The latter method

TABLE I. RAT PLASMA PROLACTIN LEVELS FOLLOWING KETAMINE, GUILLOTINING AND FROM INDWELLING CATHETERS.

Group	N	Mean ± SEM	Median	Range*	Mean coefficient of variation (%)
Ketamine	5 rats, ×10	6.0 ± 0.8	6.3	1.9–10.2	58.0
Decapitation	5 rats, ×10	6.4 ± 1.2	6.8	1.8–13.5	72.0
Catheter	5 rats, ×10	9.4 ± 1.9	8.9	2.2–19.8	63.4

* Means of each group of 5.

TABLE II. EFFECT OF KETAMINE ON PLASMA PROLACTIN LEVELS FOLLOWING RESERPINE OR AMPT.

		Plasma prolactin (ng/ml)*		
	Dose (mg/kg)	Saline	Ketamine	p
Reserpine (A)	5	18.5 ± 3.7	25.0 ± 3.1	NS
Reserpine (B)	5	21.7 ± 2.7	21.3 ± 1.7	NS
AMPT	100	15.4 ± 4.6	15.2 ± 3.9	NS

* Mean ± SEM Ketamine, 100 mg/kg ip or saline was given 3 hr 55 min (A) or 3 hr 30 min (B) following reserpine and 15 min following AMPT. Rats were sacrificed by decapitation 5 min (A) or 30 min (B) after ketamine in the reserpine-pretreated rats, and 15 min after ketamine in the AMPT-pretreated rats. All groups consisted of 5 rats.

TABLE III. EFFECT OF KETAMINE AND FLUOXETINE ON INCREASE IN PROLACTIN PRODUCED BY 5-HTP.

Pretreatment	Dose (mg/kg)	Treatment	Dose (mg/kg)	Plasma prolactin* (ng/ml)
Saline	—	Saline	—	6.4 ± 1.5
Saline	—	5-HTP	30	8.4 ± 1.5
Fluoxetine	10	Saline	—	7.5 ± 1.4
Fluoxetine	10	5-HTP	30	38.7 ± 4.6
Fluoxetine	10	Ketamine	100	6.4 ± 1.3
Ketamine	25	5-HTP	30	6.7 ± 2.3
Ketamine	50	5-HTP	30	8.6 ± 1.3
Ketamine	100	5-HTP	30	7.0 ± 1.4

* Mean ± SEM. The first injection was given 60 min before the second injection. Groups of five rats were sacrificed by decapitation 15 min after saline or 5-HTP.

of blood sampling tended to produce the highest levels and the greatest variance within a given group of 5 rats, the usual size of our control groups. These results indicate that where a single blood sample is required from a given male rat, ketamine anesthesia is acceptable. For studies in which anesthetized rats might be desirable, ketamine is clearly preferable to other anesthetics which themselves affect prolactin secretion. The reported ability of ketamine, 50 mg/kg, i.a., to lower prolactin levels, in ovariectomized estrogen-treated rats (7), if confirmed, would indicate that ketamine might affect the estrogen-stimulated prolactin secretion process and thus be less suitable for use in studies with female rats than it appears to be for male rats. The lack of effect of ketamine on prolactin secretion further documents the difference between the anesthesia produced by this agent and classical general anesthetics.

The inability of ketamine to reverse the increase in plasma prolactin levels produced by reserpine or AMPT is strong evidence that ketamine does not have direct dopamine agonist effects *in vivo* at the pituitary dopamine receptors which regulate prolactin secretion. Direct dopamine agonists such as apomorphine, bromcryptine or lysergic acid diethylamide readily reverse the increase in prolactin produced by reserpine or AMPT (17, 18 and unpublished data from this laboratory). Similarly, the inability to reverse the reserpine or AMPT-induced increase in prolactin indicates ketamine differs significantly from d-amphetamine, which has been shown to reverse the increase in prolactin secretion produced by reserpine or AMPT (19), presumably by increasing the release of dopamine from tubero-infundibular dopamine neurons or blocking its uptake. Previous studies of the effect of ketamine on dopaminergic mechanisms have been *in vitro* and have dealt with the nigro-striatal dopaminergic pathway. These differences may account for the differences between the results of those studies and this one.

The ability of fluoxetine but not ketamine to potentiate the effects of a subthreshold dose of 5-HTP on prolactin secretion indicates that ketamine is not an effective inhibitor of serotonin reuptake *in vivo* at those neurons which release the serotonin that potentiates prolactin secretion. These are believed to be the median raphe serotonergic neurons (20). However, an effect of ketamine on uptake of serotonin by other serotonergic neurons is not excluded.

The lack of effect of ketamine on the reserpine-, AMPT- and 5-HTP-induced increase in prolactin secretion indicates the suitability of ketamine for anesthesia in studies of the effect of dopaminergic and serotonergic drugs on prolactin secretion.

Summary. Mean plasma prolactin levels obtained from male rats following anesthesia with ketamine, decapitation or via indwelling venous catheters were not significantly different although a larger variance was found in the samples obtained via catheters. Ketamine, at anesthetic doses, did not affect the increases in prolactin produced by reserpine or α-methylparatyrosine. Ketamine, at various doses, did not potentiate the effect of subthreshold doses of 5-hydroxytryptophan on prolactin secretion. Thus, ketamine would appear to be a suitable anesthetic for use in studies of prolactin secretion in male rats. Further studies in female rats are required.

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