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Inhibition of Prolactin Actions in Mouse Mammary Gland Explants by *p*-Bromphenacyl Bromide, a Phospholipase A_2 Inhibitor (40552)[1]

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p-Bromphenacyl bromide (BPB) has been shown to be an effective inhibitor of phospholipase A_2 activity (1). Since we have reported earlier that certain of the actions of prolactin in the mammary gland may be triggered by a stimulation of membrane associated phospholipase A_2 (2, 3), the effects of BPB on the actions of prolactin on RNA and casein synthesis in mammary gland explants have been tested.

Materials and methods. Pregnant Swiss–Webster mice were used for experimentation 10–14 days after Day 1 of pregnancy; they were purchased from Spartan Research Animals Inc., Haslett, Mich. Ovine prolactin (NIH-P-S-13) was obtained from the National Institute of Arthritis and Metabolic Diseases. Other substances were purchased from the following sources: Hydrocortisone from Charles Pfizer and Company; Nystatin from E. R. Squibb and Sons, Inc.; Medium 199, penicillin, and streptomycin from KC Biological Inc.; [5,6-^{3}H]uridine (41.3 Ci/mmole), [4,5-^{3}H]leucine (51.6 Ci/mmole), and [choline-methyl-^{14}C]phosphatidyl choline (50 mCi/mmole) from the New England Nuclear Corp.; procine insulin from the Eli Lilly Company; bee venom phospholipase A_2 from Sigma Chemical Company; and *p*-bromphenacyl bromide (BPB) from Aldrich Chemical Company.

Incubations and preparation of mammary gland explants were accomplished by methods employed earlier (4). Briefly, explants (3–5 mg each) were prepared for incubation and three explants were placed on siliconized lens paper floating on 2 ml Medium 199. When the effect of BPB on the insulin stimulation of RNA synthesis was to be studied, the tissues were cultured for 24 hr in the absence of hormones. Insulin (2.5 μg/ml) and/or BPB were then added to the culture medium and incubation was continued for 6 hr. [^{3}H]Uridine was added to the culture medium for the final hour of incubation. The amount of [^{3}H]uridine incorporated into RNA was then assessed as described earlier (4). If the effects of BPB on the prolactin stimulation of RNA and casein syntheses were to be studied, the explants were initially cultured for 2 days with Medium 199 containing insulin (2.5 μg/ml) and hydrocortisone (1 μg/ml). Prolactin (2.5 μg/ml) and/or BPB were then added to the culture medium and incubations were continued for 6 (RNA experiments) or 14 hr (casein experiments). The rates of [^{3}H]uridine incorporation into RNA and [^{3}H]leucine incorporation into a casein-rich protein fraction were used as indices of the rates of RNA and casein syntheses, respectively; these labeled compounds were added to the culture media (1 μCi/ml) 1 hr prior to termination of the incubations. Methods used to isolate and quantitate the labeled macromolecules were described earlier (4). When BPB was added to the culture medium, it was added in ethanol such that the final concentration of ethanol in the medium did not exceed 0.125%. This concentration of ethanol does not affect the basal rates of RNA or casein synthesis in the explants; nor does it affect the magnitude of the responses to prolactin (unpublished observations).

Phospholipase A_2 activity was assessed by methods employed earlier (3). For the experiment presented in this report, phosphatidyl choline labeled with [^{14}C]choline was incubated with bee venom phospholipase A_2 for 2 hr. The ratio of [^{14}C]lysolecithin to labeled phosphatidyl choline was then calculated (3).

Statistical comparisons were made with Student's *t* test or the multiple *t* test.

Results. Various concentrations of BPB were used to modify the prolactin-stimulated rates of [^{3}H]uridine incorporation into RNA (Table I). The response to prolactin was re-

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duced at a BPB concentration of 0.05 m*M* and abolished with 0.125 m*M* BPB. The inhibitory effect of BPB on casein synthesis was a bit more sensitive in that BPB concentrations of 0.05 m*M* and above abolished the prolactin response. In addition, at 0.25 m*M*, BPB significantly reduced the basal rate of [^{3}H]leucine incorporation into casein.

Insulin, like prolactin, stimulates the rate of [^{3}H]uridine incorporation into RNA in mammary gland explants of mice (5). The specificity of the inhibition of the prolactin responses by BPB was therefore tested by determining whether the BPB would affect the insulin stimulation of RNA synthesis. Table III shows that insulin stimulated the rate of [^{3}H]uridine incorporation into RNA in the presence of all BPB concentrations of up to 0.25 m*M*. The insulin stimulation was observed at the 0.25 m*M* BPB concentration, though the basal rate of [^{3}H]uridine incorporation was significantly reduced by this concentration of BPB.

The dose–response inhibition of phospholipase A_2 activity is shown in Table IV. BPB at 0.05 m*M* was the lowest concentration at which a small but significant inhibition of phospholipase A_2 activity was observed; higher concentrations of BPB had greater inhibitory effects.

TABLE I. EFFECT OF *p*-BROMOPHENACYL BROMIDE ON THE PROLACTIN STIMULATION OF RNA SYNTHESIS IN MOUSE MAMMARY GLAND EXPLANTS*

p-Bromophenacyl bromide concentration (m*M*)	[^{3}H]Uridine incorporation into RNA (dpm/μg RNA)		
	Control (C)	Prolactin (Prl)	*P* (C vs Prl)
0	39.6 ± 2.1	73.0 ± 4.2	<0.01
0.0125	37.8 ± 1.9	73.9 ± 2.8	<0.01
0.025	38.5 ± 1.3	77.0 ± 5.0	<0.01
0.050	37.5 ± 1.6	56.1 ± 2.1**	<0.01
0.125	37.0 ± 2.3	43.0 ± 3.2**	NS

* Explants were cultured for 2 days with insulin plus hydrocortisone. Prolactin and/or BPB were then added to certain flasks and incubation was continued for 6 hr. 0.5 μCi/ml [^{3}H]uridine was added to the medium 1 hr prior to termination of the incubations. Numbers represent the means ± SE of explants from seven flasks.

** Prolactin response is significantly less ($P < 0.01$) than responses in the presence of lower BPB concentrations.

TABLE II. EFFECT OF *p*-BROMPHENACYL BROMIDE ON THE PROLACTIN STIMULATION OF CASEIN SYNTHESIS IN MOUSE MAMMARY GLAND EXPLANTS*

p-Bromphenacyl bromide concentration (m*M*)	[^{3}H]Leucine incorporation into casein (dpm/mg wet tissue wt)		
	Control**	Prolactin**	*P* (C vs Prl)
0	388 ± 12^a	601 ± 46^c	<0.01
0.01	410 ± 23^a	634 ± 38^c	<0.01
0.05	405 ± 26^a	497 ± 41^d	NS
0.10	398 ± 35^a	344 ± 24^e	NS
0.25	103 ± 9^b	101 ± 12^f	NS

* Explants were cultured for 2 days with insulin plus hydrocortisone. Prolactin and/or BPB was then added to certain flasks and incubation was continued for 14 hr. 1 μCi/ml [^{3}H]leucine was added to the medium 1 hr prior to termination of the incubations. Numbers represent the mean ± SE of explants from seven flasks.

** The following experimental combinations are significantly different ($P < 0.01$); *a* vs *b*, *c* vs *d*, *c* vs *e*, *c* vs *f*, *d* vs *e*, *d* vs *f*, and *e* vs *f*.

TABLE III. EFFECT OF *p*-BROMPHENACYL BROMIDE ON THE INSULIN STIMULATION OF RNA SYNTHESIS IN MOUSE MAMMARY GLAND EXPLANTS*

p-Bromphenacyl bromide concentration (m*M*)	[^{3}H]Uridine incorporation into RNA (dpm/μl RNA)		
	Control	Insulin	*P* (C vs Prl)
0	106 ± 6	242 ± 11	<0.01
0.01	101 ± 7	240 ± 14	<0.01
0.05	108 ± 4	249 ± 15	<0.01
0.1	105 ± 7	221 ± 4	<0.01
0.25	26 ± 3**	45 ± 3**	<0.01

* Explants were cultured for 1 day in the absence of hormones. Insulin and/or BPB were then added to certain flasks and incubation was continued for 6 hr. 1 μCi/ml [^{3}H]uridine was added to the medium 1 hr prior to termination of the incubations. Numbers represent the mean ± SE of explants from seven flasks.

** Significantly less ($P < 0.01$) than in the presence of lower concentrations of BPB.

TABLE IV. EFFECT OF *p*-BROMPHENACYL BROMIDE ON PHOSPHOLIPASE A_2 ACTIVITY

p-Bromphenacyl bromide concentration (m*M*)	Phospholipase A_2 activity	
	(0.16 mU bee venom)	*P* (C vs BPB)
0	0.471 ± 0.009*	
0.025	0.447 ± 0.013	NS
0.050	0.431 ± 0.004	<0.01
0.10	0.437 ± 0.013	<0.05
0.25	0.382 ± 0.010	<0.01
0.50	0.340 ± 0.016	<0.01
1.00	0.261 ± 0.009	<0.01

* Numbers represent the ratio of [^{14}C]lysophosphatidyl choline to [^{14}C]phospatidyl choline after a 120-min incubation period. Numbers represent the mean ± SE of quadruplicate determinations.

Discussion. These studies show that BPB, an inhibitor of phospholipase A_2 activity, clearly interferes with the actions of prolactin on RNA and casein synthesis in mammary gland explants. Since BPB did not affect the insulin stimulation of RNA synthesis in the explants, the specificity of the inhibition of the prolactin responses by BPB is suggested. These observations, therefore, support the hypothesis that the actions of prolactin on RNA and casein synthesis in the mammary gland require a prior activation of phospholipase A_2 by prolactin. BPB at 0.25 m*M* was also observed to decrease the basal rate of [^{3}H]-leucine incorporation into casein. This may be related to the inhibition of basal phospholipase A_2 activity, or it may be due to some other nonspecific action of this drug.

The inhibition of prolactin's actions on RNA and casein synthesis occurred at BPB concentrations (0.05 m*M*) which did not have very large inhibitory effects on phospholipase A_2 activity. However, since BPB is a lipid-soluble substance, it is indeed possible that the BPB accumulates in the plasma membranes of the cultured cells. The membrane associated phospholipase A_2 could therefore be exposed to much higher concentrations of this drug than those employed in the enzyme assay system. Such a possibility is suggested by the observation that quinacrine, another lipid-soluble drug, is concentrated in cellular membranes (6, 7). It is also of interest that quinacrine, another inhibitor of phospholipase A_2, has recently been shown to abolish the effects of prolactin on RNA and casein synthesis in mouse mammary gland explants (8).

Summary. The stimulatory actions of prolactin on RNA and casein synthesis in mouse mammary gland explants were shown to be significantly reduced or abolished by incubation with *p*-bromphenacyl bromide (BPB) at concentrations of 0.05 m*M* or greater. Since BPB has been shown to be an inhibitor of phospholipase A_2 activity, the inhibition of prolactin's actions by BPB provides further support for the proposal that at least certain of the actions of prolactin in the mammary gland may be initiated by a stimulation of membrane-associated phospholipase A_2 activity.

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