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Stimulation of Prolactin Release by the Bicarbonate Ion (40581)[1]

STEVEN W. J. LAMBERTS[2] AND ROBERT M. MACLEOD

Department of Internal Medicine, University of Virginia School of Medicine, Charlottesville, Virginia 22908

Evidence has been presented that dopamine is a hypothalamic factor responsible for the direct inhibition of prolactin (PRL) release (1–3). Physiological factors which stimulate PRL release are less well established. This study shows that the bicarbonate ion stimulates PRL release by the pituitary gland *in vitro* and that increasing the bicarbonate concentration of the incubation medium decreases dopamine's inhibitory effect on PRL release.

Materials and methods. Groups of three or four flasks each containing three hemipituitary glands from mature female Wistar–Furth rats in 1 ml Krebs–Ringer buffer containing 10 μCi [4,5-^{3}H]leucine (30 Ci/mmole) were placed in a Dubnoff shaker under 95% O_2, 5% CO_2 and incubated for 5 hr at 37°. The concentration of bicarbonate in the medium was changed by adding $NaHCO_3$ and then adjusting the pH to 7.4 by adding NaOH. Aliquots of pituitary homogenate and/or incubation medium were subjected to polyacrylamide gel electrophoresis as described in MacLeod *et al.* (4). The location of PRL on the gels was ascertained by subjecting NIH reference hormone to the same electrophoretic separation. The PRL content of the incubation medium was measured by a double-antibody radioimmunoassay using materials and protocols supplied by the NIAMDD Rat Pituitary Hormone Distribution program. The results are expressed as mean ± SEM and statistical analysis was performed by analysis of variance.

Results. The effect of varying the medium bicarbonate concentration of pituitary PRL release was studied initially by increasing the $NaHCO_3$ concentration of a Krebs–Ringer buffer from 0 to 24.6 mM (Fig. 1). A progressive stimulation of PRL release was observed. In another experiment, increasing the incubation medium bicarbonate concentration from 22 to 32 mM led to a further 92% increase in the release of PRL (data not shown). Variations in the NaCl concentration of the buffer did not affect PRL release.

In addition to direct stimulation of PRL release, increasing the incubation medium bicarbonate concentration decreased the inhibitory effect of dopamine on PRL release (Fig. 2). In incubation medium with bicarbonate concentration of 14 mM, 500 nM dopamine inhibited the release of [^{3}H]PRL 74% ($P < 0.01$) and of radioimmunoassayable PRL (RIA-PRL) 46% ($P < 0.01$), whereas the same concentration of dopamine caused only 38% inhibition (NS) of [^{3}H]PRL release and 27% inhibition (NS) of RIA-PRL release when added to medium containing 22 mM bicarbonate. The highly significant *in vitro* inhibitory effects of 10 nM ergocryptine on pituitary [^{3}H]PRL and RIA-PRL release in buffers containing 15 and 22 mM bicarbonate were essentially the same (Fig. 3). Although ascorbate had no effect on the bicarbonate-mediated release of prolactin, the addition of 0.5 μg ascorbate to incubation medium containing 22 mM bicarbonate resulted in 500 nM dopamine inhibiting [^{3}H]PRL release by 78% (data not presented).

The effect of bicarbonate on the lactotroph is confined to a stimulating effect on the release of PRL. The total newly synthesized PRL, calculated by taking the sum of [^{3}H]-PRL in the medium and in the pituitary gland, did not change when the bicarbonate concentration of the incubation buffer increased from 14 to 22 mM (Figs. 2 and 3) and from 22 to 32 mM.

Discussion. It has been shown before that changes in the ionic environment of the pituitary gland *in vitro* greatly influence the synthesis and release of several pituitary hormones (5–8). Variations in the potassium con-

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[2] On leave from Department of Internal Medicine (III) and Clinical Endocrinology, University Hospital Dijkzigt, Erasmus University, Rotterdam, The Netherlands.

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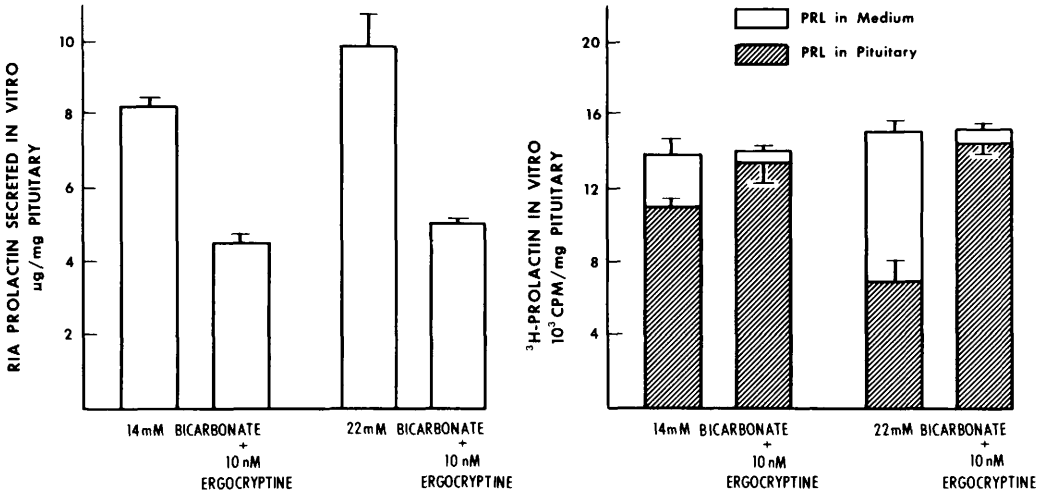

FIG. 1. The effect of an increase in the bicarbonate concentration on the release of [^{3}H]PRL by the pituitary gland *in vitro*. Three hemipituitary glands from normal female rats were incubated for 5 hr in 1 ml Krebs-Ringer buffer containing [^{3}H]leucine. The incubation medium was subjected to polyacrylamide gel electrophoresis. The bands containing [^{3}H]PRL were counted. Each group consisted of four flasks.

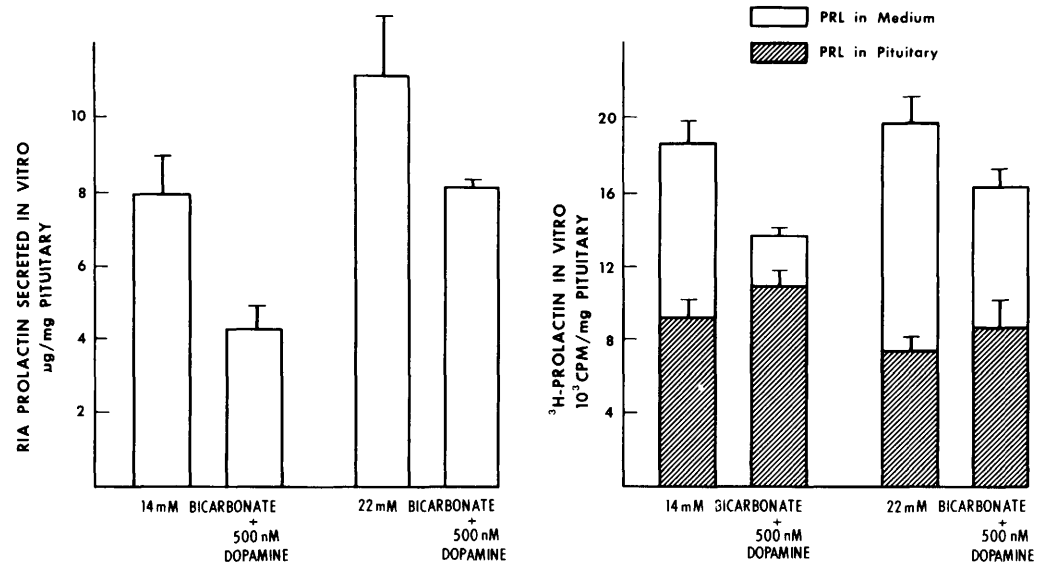

FIG. 2. The effect of the increase of bicarbonate concentration in the incubation medium on the inhibitory effect of 500 n*M* dopamine on synthesis and release of PRL. Female rat pituitary glands incubated as described in Fig. 1. PRL in the incubation medium was measured by RIA.

centration of the medium mainly influence the release of growth hormone (6), ACTH (7), and LH (8), while a 500% increase in the potassium concentration had no effect on the release of PRL by the pituitary gland *in vitro* (5). A 500% increase in the calcium concentration of the medium was reported to increase the synthesis and release of PRL without affecting the synthesis of growth hormone (5).

The selective effect on PRL release of variations in the bicarbonate concentration in the medium bathing rat pituitary glands, without affecting PRL synthesis, was unexpected. The concentrations of bicarbonate used (14–32 m*M*) cover the physiological

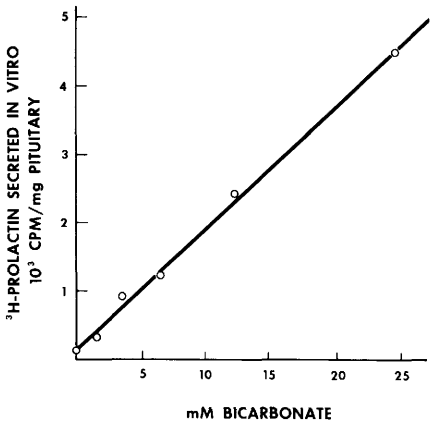

FIG. 3. The absence of an effect of the increase of bicarbonate concentration in the incubation medium on the inhibitory effect of 10 n*M* ergocryptine on synthesis and release of PRL. Female rat pituitary glands incubated as described in Fig. 1.

range, including that found in pathological conditions in man. The decreased inhibitory effect of dopamine on PRL release *in vitro* in the presence of increased bicarbonate concentrations could be caused by decreased access to the dopamine receptor on pituitary membranes or by decreased availability of unmetabolized dopamine in this medium. The observations that ascorbate restored the dopamine effect on PRL release and that an increase in the bicarbonate concentration did not influence ergocryptine-mediated inhibition of PRL release favors the latter explanation.

Since the dopamine-mediated inhibition of PRL release is less well demonstrated at high bicarbonate concentrations than at low bicarbonate levels, we suggest that the dopaminergic system and bicarbonate in the blood may well interact in the pituitary gland to regulate PRL release. This might partly explain the non-R.E.M. sleep-related PRL release surges in man (9). During non-R.E.M. sleep in man, the blood CO_2 tension increases (9) and that may be associated with the sleep-related increase in serum prolactin.

Summary. The bicarbonate ion was found to stimulate the *in vitro* release of RIA-PRL and [^{3}H]PRL. Dopamine, as usual, inhibited the *in vitro* release of prolactin; however, the effects of the catecholamine were less evident at high molar concentrations of bicarbonate than at lower ones. Sodium ascorbate addition to high bicarbonate concentration increased the effectiveness of dopamine to inhibit prolactin. Ergocryptine decreased greatly the *in vitro* release of prolactin, and varying the bicarbonate concentration had no effect on this phenomenon. The results suggest that bicarbonate may interact with dopamine in regulating the physiological release of prolactin.

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