PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 162, 31-33 (1979)

# Effects of Nifedipine on Prolactin, Growth Hormone, and Luteinizing Hormone Release by Rat Anterior Pituitary *in Vitro* (40612)

JIRO TAKAHARA, SHO YUNOKI, YUKIO YAMANE, JIRO YAMAUCHI, WATARU YAKUSHIJI, NOBUKO KAGEYAMA, KAZUYUKI FUJINO, AND TADASHI OFUJI

*Third Department of Internal Medicine, Okayama University School of Medicine, Okayama 700, Japan*

It is well known that $Ca^{2+}$ plays an important role in hormone release from the anterior pituitary (1–8); however, the mechanism of $Ca^{2+}$ action on the anterior pituitary cells remains to be clarified. Douglas *et al.* (9, 10) proposed that hormone release from the neurohypophysis and adrenal medulla is initiated by depolarization of the cell plasma membrane. Depolarization induces a permeability change in the plasma membrane resulting in an influx of $Ca^{2+}$ into the cells, and this influx may then initiate the intracellular hormone-releasing mechanism.

In the present study, we investigated the effects of nifedipine, a $Ca^{2+}$ blocker, on *in vitro* release of prolactin, GH, and LH from the anterior pituitary.

*Materials and methods.* Male Sprague–Dawley rats, weighing 180–200 g, were used. The animals were decapitated, and the anterior pituitary was immediately removed and bisected to equal halves. The two halves of each pituitary were placed in a beaker with 1 ml of Krebs–Ringer bicarbonate medium containing 0.25% bovine serum albumin (KRBG–BSA). After two 1-hr preincubation periods in a metabolic incubator at 37° under 95% $O_2$ and 5% $CO_2$ gas, the medium was replaced with fresh KRBG–BSA (1 ml) and incubated for 1 hr (first incubation). The incubation medium was collected. Then 1 ml of KRBG–BSA containing the test material was added to the beaker and incubated for another 1 hr (second incubation), and the incubation medium was collected. The amount of pituitary hormone released during the second 1-hr incubation was compared with that released during the first 1-hr incubation period and expressed as a percentage of that released in the first hour.

Nifedipine (Adalat, Bayer Pharmacol. Co.) was dissolved in a vehicle of 15% polyethylene glycol 400, 15% ethanol, and 70% distilled water in concentrations of 100, 10, and 1 μg/ml: Each solution was diluted 10 fold with KRBG–BSA, and then 0.1 ml of solution containing nifedipine was added to 0.9 ml KRBG–BSA medium. The vehicle was also diluted with KRBG–BSA 10-fold, and 0.1 ml of the diluted vehicle was added to samples without nifedipine. Prolactin, GH, and LH levels were determined in each incubation medium by specific radioimmunoassays using rat prolactin, GH, and LH radioimmunoassay kits supplied by NIAMDD. Duncan's new multiple range test was used for comparing the mean release of prolactin, GH, or LH in each beaker.

*Results. Nifedipine on prolactin release in vitro.* Nifedipine at 10, 100, and 1000 ng/ml progressively inhibited prolactin release in a dose-related manner (Table I). TRH at 100 ng/ml appeared to increase prolactin release, but this effect was not statistically significant compared to the control. The slight stimulation of prolactin release induced by TRH was also inhibited by nifedipine.

*Nifedipine on GH release in vitro.* Nifedipine at 10, 100, or 1000 ng/ml had no effect on GH release. TRH at 100 ng/ml significantly increased GH release compared to the control. Nifedipine at 1 μg/ml blocked the TRH-induced GH release.

*Nifedipine on LH release in vitro.* Neither nifedipine nor TRH affected LH release from the anterior pituitary. LH-RH at 100 ng/ml caused a fivefold increase in LH release. Nifedipine at 1 μg/ml significantly blocked the release of LH induced by LH-RH.

*Discussion.* Nifedipine at 10, 100, or 1000 ng/ml inhibited the release of prolactin, but not that of GH or LH, from rat anterior pituitary tissue. Nifedipine at 100 or 1000 ng/ml blocked not only the TRH-induced GH



0037-9727/79/090031-03$01.00/0

TABLE I. EFFECTS OF NIFEDIPINE ON PROLACTIN, GH AND LH RELEASE IN VITRO RAT PITUITARY TISSUE

| Treatment | Dose (ng/ml) | No. beakers | (2nd incubation/1st incubation) × 100 | | |
|---|---|---|---|---|---|
| | | | Prolactin | GH | LH |
| (1) Control | — | 4 | 97.0 ± 6.8[α] | 102.6 ± 5.0 | 114.8 ± 8.6 |
| (2) Nifedipine | 10 | 4 | 81.9 ± 16.4 | 101.9 ± 12.0 | 106.9 ± 13.9 |
| (3) Nifedipine | 100 | 4 | 52.2 + 4.1** | 86.4 ± 12.4 | 122.3 ± 7.5 |
| (4) Nifedipine | 1000 | 4 | 41.4 ± 5.7** | 104.1 ± 4.3 | 118.3 ± 16.8 |
| (5) TRH | 100 | 4 | 124.3 ± 19.6 | 144.1 ± 4.8* | 109.6 ± 8.8 |
| (6) Nifedipine<br>TRH | 100<br>100 | 4 | 88.5 ± 5.6 | 133.7 ± 6.3 | 113.2 ± 4.8 |
| (7) Nifedipine<br>TRH | 1000<br>100 | 4 | 67.2 ± 1.0[α] | 104.5 ± 8.1[β] | 108.4 ± 8.9 |
| (8) LH-RH | 100 | 4 | 85.2 ± 7.8 | 117.7 ± 13.1 | 562.7 ± 61.8** |
| (9) Nifedipine<br>LH-RH | 1000<br>100 | 4 | 41.7 ± 3.8 | 102.8 ± 4.8 | 398.8 ± 44.3**[γ] |

[α] Mean ± SEM.
* $p < 0.05$ vs control.
** $p < 0.01$ vs control.
[α] $p < 0.01$ vs (5).
[β] $p < 0.05$ vs (5).
[γ] $p < 0.05$ vs (8).

but the LH-RH-induced LH release as well. Nifedipine at 1000 ng/ml also inhibited the slight stimulation of prolactin release induced by TRH. Nifedipine has been shown to have a strong blocking effect on excitation–contraction coupling in myocardium without inhibiting the electrical excitation process (11). It inhibits excitation–contraction coupling by blocking $Ca^{2+}$ influx from the extracellular space.

It is well known that $Ca^{2+}$ is involved in the releasing mechanism of pituitary hormones. Removal of $Ca^{2+}$ from the incubation medium inhibits their release. Samli and Geshwind (2) reported the stimulatory effect of high $K^+$ medium on LH secretion and demonstrated the $Ca^{2+}$ requirement for LH-releasing action in response to both high $K^+$ medium and hypothalamic extracts. Wakabayashi *et al.* (3) found that $Ca^{2+}$ uptake initiates the release of gonadotropins. Vale *et al.* (1) also reported that $Ca^{2+}$ is required for TRH-induced secretion.

An interesting finding was that nifedipine at 10 to 1000 ng/ml did not affect the release of GH or LH, but that prolactin release was blocked by nifedipine in a dose-related manner. MacLeod and Fontham (5) found that the absence of $Ca^{2+}$ from Krebs–Ringer bicarbonate medium greatly diminished prolactin synthesis and release. GH synthesis and release were also decreased by incubation in $Ca^{2+}$-free medium, but the relative decrease was greater with prolactin than with GH. They also found that a fivefold higher $Ca^{2+}$ concentration increased the synthesis and release of prolactin without affecting the synthesis of GH. Our results and the data of Macleod and Fontham indicate that the mechanism of prolactin release from the anterior pituitary may be more closely involved with $Ca^{2+}$ influx from extracellular fluid and in stimulus–secretion coupling than the release of GH or LH.

Eto *et al.* (12) reported that verapamil, a $Ca^{2+}$ blocker, did not inhibit the basal release of ACTH, GH, or TSH. They also reported that verapamil had no effect on ACTH release induced by crude hypothalamic extracts or synthetic lysine vasopressin, and that verapamil did not inhibit the TSH release induced by synthetic TRH or the GH release induced by crude hypothalamic extract. They thus concluded that hypothalamic releasing factors did not affect the process of stimulus–secretion coupling by promoting a specific calcium influx. Zimmerman and Fleisher (7) reported that the ACTH release induced by vasopressin decreased after incubation in $Ca^{2+}$-free medium.

Wakabayashi *et al.* (3) also reported that the effects of releasing factors on the LH and FSH response were only partially inhibited in $Ca^{2+}$-free medium, while the action of high $K^+$ was completely abolished. In this paper, nifedipine at relative large doses completely

inhibited the release of TRH-induced GH and partially, but significantly, prevented the LH-RH-induced LH release, and also nifedipine at a large dose inhibited the release of the TRH-induced prolactin release.

*Summary.* The release of prolactin, GH, and LH was monitored by radioimmunoassay in rat anterior pituitary tissue incubated in Krebs–Ringer bicarbonate medium. Nifedipine, a strong $Ca^{2+}$ blocker, at 10, 100, and 1000 ng/ml inhibited the release of prolactin in a dose-related manner, but did not inhibit the release of GH and LH. This drug also blocked the TRH-induced GH release and the LH-RH-induced LH release. These results indicate that the release of prolactin may be more closely tied to $Ca^{2+}$ influx from extracellular fluid and with stimulus–secretion coupling than the release of GH and LH and that $Ca^{2+}$ may also play a role in the release of anterior pituitary hormones induced by releasing hormones.

1. Vale, W., Burgus, R., and Guillemin, R., Experientia **23,** 855 (1967).
2. Samli, M., and Geshwind, I. I., Endocrinology **82,** 225 (1968).
3. Wakabayashi, K., Ibrahim, A. K., and McCann, S. M., Endocrinology **85,** 1046 (1969).
4. Kraicer, J., Milligan, J. V., Gosbee, J. L., and Conrad, R., Endocrinology **85,** 1144 (1969).
5. MacLeod, R. M., and Fontham, E. H., Endocrinology **86,** 863 (1970).
6. Parsons, J. A., J. Physiol. (London) **210,** 973 (1970).
7. Zimmerman, G., and N. Fleisher, Endocrinology **87,** 426 (1970).
8. Williams, J. A., J. Physiol. (London) **260,** 105 (1976).
9. Douglas, W. W., and Poisner, A. M., J. Physiol. (London) **172,** 1 (1964).
10. Douglas, W. W., and Rubin, R. P., J. Physiol. (London) **167,** 288 (1963).
11. Fleckenstein, A., Trithart, H., Doring, H.-J., and Byon, K. Y., Arzneim-Forsch. **22,** 22 (1972).
12. Eto, S., Wood, J. M., Hutchins, M., and Fleisher, N., Amer. J. Physiol. **226,** 1315 (1974).

Received December 22, 1978. P.S.E.B.M. 1979, Vol. 162.