EFFECTS OF ISOBUTYLMETHYLXANTHINE ON CYCLIC NUCLEOTIDE LEVELS IN RAT TESTIS

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ABSTRACT

The effects of 3-isobutyl-1-methylxanthine (IBMX) on levels of cyclic nucleotides and testosterone were studied in rat testis *in vitro*. IBMX at 1.0 mM increased cyclic GMP levels about 17-fold. Levels of cyclic AMP and testosterone were also increased, but slightly. The increase in cyclic GMP was mainly in Leydig cell fraction. The effect of IBMX on testicular cyclic AMP and testosterone is in the same order as that of theophylline, whereas the effect on cyclic GMP is far larger than that of theophylline. IBMX did not interfere with *in vitro* production of cyclic AMP and testosterone by human chorionic gonadotropin (HCG). The results suggest that at least two separate processes are operating in the rat testis for the hydrolysis of cyclic AMP and cyclic GMP, one of which is strongly influenced by IBMX.

It has been proposed that cyclic GMP acts intracellularly to elicit responses which oppose to, or differ from, those of cyclic AMP (4-6). However, neither specific effects nor specific hormonal regulations of cyclic GMP in rat testis have been reported.

Hydrolysis catalyzed by cyclic nucleotide phosphodiesterase (PDE) is the only known pathway for biological degradation of cyclic nucleotides in eukaryotic cells (13), and inhibitors of cyclic nucleotide PDE are often used as pharmacological tools to demonstrate the role of cyclic nucleotides as second messengers in various physiological phenomena (8). Several PDE isoforms have been isolated from a variety of tissues (1, 3, 11, 14, 16, 17), and most of the documented PDE inhibitors are rather non-selective as inhibitors of the multiple PDEs (2). In the case of 3-isobutyl-1-methylxanthine (IBMX), which is one of the most potent PDE inhibitors (2, 13), different results were reported depending on the tissues examined, with regard to its selectivity in the inhibition of cyclic GMP-PDE (7, 12, 13, 18).

In the present study, we examined the effects of IBMX on cyclic nucleotides levels in rat testis tissue, to clarify its selectivity to PDEs, and to study possible contribution of cyclic GMP to trophic hor-

mone effects.

MATERIALS AND METHODS

Three-month-old Wistar rats, weighing 250 to 300 g, were anesthetized with ether. The testes were removed quickly, decapsulated and sectioned into slices of 50 mg each. Each slice was put into a test tube filled with 0.5 ml of Krebs-Ringer-bicarbonate buffer (pH 7.4) containing 2 mg/ml glucose (KRBG) and preincubated for 30 min at 34°C. Following preincubation, each testis slice was incubated at 34°C for 3 h in 0.5 ml of KRBG containing various concentrations of IBMX or theophylline in the presence or absence of human chorionic gonadotropin (HCG). Preincubation and incubation were performed with constant shaking (120 cycle/min) in an atmosphere of 95% oxygen and 5% carbon dioxide. At the end of incubation, aliquots of the medium were taken for measurement of cyclic AMP, cyclic GMP and testosterone.

In another experiment, decapsulated testes were sectioned into slices of 80 mg. Each slice was separated into fractions of seminiferous tubules and stroma (Leydig cells) by the forceps in the cooled KRBG. The effects of IBMX on cyclic AMP,

Fig. 1 Effects of IBMX on cyclic AMP, cyclic GMP and testosterone levels in rat testicular slices in KRBG solution *in vitro*. Values represent the mean+SD for three testis slices.

cyclic GMP and testosterone in each fraction were examined in the same way as described above.

Both cyclic AMP and cyclic GMP were determined by a radioimmunoassay method (15) as modified by ourselves (9), and testosterone according to Nieschlag *et al.* (10).

RESULTS

IBMX at 0.5 mM or higher caused a marked increase in cyclic GMP and a slight increase in cyclic AMP levels in rat testis tissue *in vitro* (Fig. 1). In the presence of IBMX, testosterone production was also stimulated above the control level, probably due to the slightly elevated level of cyclic AMP. Maximal effects of IBMX on both cyclic GMP and cyclic AMP were observed at the concentration of 1 mM, where the level of cyclic GMP was

Fig. 2 Effects of theophylline on cyclic AMP, cyclic GMP and testosterone levels in rat testicular slices in KRBG solution *in vitro*. Values represent the mean+SD for three testis slices.

1,700% and cyclic AMP was 200% of the respective controls.

The effects of theophylline were studied as a standard PDE inhibitor. Theophylline at 5 mM or 10 mM increased cyclic AMP, cyclic GMP and testosterone to a level only 2 to 3 times greater than the respective control levels (Fig. 2). Increase in cyclic GMP level was much lower compared to IBMX administration.

To study the specificity of IBMX, Leydig cell and seminiferous tubule fractions were separated and incubated in the presence of IBMX. As shown in Fig. 3, IBMX increased cyclic AMP and cyclic GMP in the Leydig cell fraction. Only a marginal increase was observed in the seminiferous tubule fraction.

Effects of IBMX on HCG-mediated cyclic nucleotide increase and testosterone production were

Fig. 3 Effects of IBMX on cyclic AMP, cyclic GMP and testosterone levels in separated seminiferous tubules and the Leydig cell fraction of the rat testis in KRBG solution *in vitro*. Values represent the mean + SD for three testis slices.

also examined. HCG increased cyclic AMP and testosterone production, but did not increase cyclic GMP level (Fig. 4). When the testis was incubated in a medium containing 0.1–1.0 mM of IBMX, stimulation of cyclic AMP production by HCG was augmented. IBMX increased the cyclic GMP level irrespective of the presence or absence of HCG (Fig. 4). IBMX did not interfere with the HCG-induced stimulation of testosterone production.

DISCUSSION

The studies reported here showed that IBMX markedly increased cyclic GMP levels in the rat testis *in vitro*, especially in the Leydig cell fraction, while its effect on cyclic AMP levels was slight. The different effects of IBMX on testicular cyclic GMP and cyclic AMP suggest the presence of at least two different cyclic nucleotide PDEs in rat testis. In fact, Purvis and Rui (11) demonstrated several distinct forms of cyclic nucleotide PDE in the rat testis. Similar observations were made in other tissues (1, 3, 14, 16, 17). The present results obtained with rat testis indicate, in contrast to theophylline, IBMX is a selective inhibitor of cyclic GMP-PDE. A similar selectivity was reported in the cat retina (12), although IBMX is

Fig. 4 Effects of HCG *in vitro* on cyclic AMP, cyclic GMP and testosterone production in the presence of IBMX. The testis slices were incubated in KRBG solution. Values represent the mean + SD for three testis slices.

generally thought to be a non-selective inhibitor (7, 13, 18). The effect of IBMX, therefore, seems to differ depending on the tissues examined. Our results indicate separate factors regulating the degradation of cyclic AMP and cyclic GMP in rat testis.

Our present results support the observations by Purvis and Rui (11) that a PDE, relatively specific for cyclic GMP, is present in and characteristic of the endocrine element of the testis, i.e. Leydig cells as opposed to germinal cells. They stressed that a higher capacity for hydrolyzing cyclic GMP than cyclic AMP appears to be a characteristic of endocrine elements such as Leydig cells and pituitary gland. They also speculate a possible contribution of cyclic GMP in trophic hormone effects. Our data, however, showed no difference in HCG-stimulated testosterone production by the rat testis in the presence of high or low concentration of cyclic GMP.

In summary, it is concluded that multiple PDE activities are present in rat testis, one of which is cyclic GMP-PDE activity, and IBMX is a potent inhibitor of it. IBMX may be a tool for analyzing effects of cyclic GMP in the rat testis, although further data are required to establish precisely the mechanism of IBMX effect on testicular cyclic nucleotide metabolism.

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REFERENCES

1. BEAVO J. A., HANSEN R. S., HARRISON S. A., HURWITZ R. L., MARTINS T. J. and MUMBY M. C. (1982) Identification and properties of cyclic nucleotide phosphodiesterases. *Mol. Cell. Endocrinol.* **28**, 387-410
2. CHASIN M. and HARRIS D. N. (1976) Inhibitors and activators of cyclic nucleotide phosphodiesterase. *Adv. Cyclic Nucleotide Res.* **7**, 225-264
3. DETERRE P., BIGAY J., FORQUET F., ROBERT M. and CHABRE M. (1988) cGMP phosphodiesterase of retinal rods is regulated by two inhibitory subunits. *Proc. Natl. Acad. Sci. USA* **85**, 2424-2428
4. FISCUS R. R. and MURAD F. (1988) cGMP-dependent protein kinase activation in intact tissues. *Meth. Enzymol.* **159**, 150-158
5. GEORGE W. J., POLSON J. B., O'TOOLE A. G. and GOLDBERG N. D. (1970) Elevation of guanosine 3',5'-cyclic phosphate in rat heart after perfusion with acetylcholine. *Proc. Natl. Acad. Sci. USA* **66**, 398-403
6. GOLDBERG N. D., O'DEA R. F. and HADDOX M. K. (1973) Cyclic GMP. *Adv. Cyclic Nucleotide Res.* **3**, 155-223
7. HASHIMOTO H., ISHII K., SATOH K. and TAIRA N. (1987) Comparison of vasodilator effects of DN-9693, a selective inhibitor of cyclic AMP phosphodiesterase, and isobutylmethylxanthine, a non-selective one, in dogs. *Jap. J. Pharmacol.* **44**, 405-412
8. HIDAKA H. and ENDO T. (1984) Several inhibitors of three forms of cyclic nucleotides phosphodiesterase-basic and potential clinical applications. In *Advances in Cyclic Nucleotide and Protein Phosphorylation Research* (ed. GREENGARD P. and ROBISON G. A.) Raven Press, New York, pp. 245-259
9. MIYACHI Y., MIZUCHI A. and SATO K. (1977) Preparation of iodinated cyclic GMP derivatives by a lactoperoxidase method. *Anal. Chem.* **77**, 429-435
10. NIESCHLAG E. and LORIAUX D. L. (1972) Radioimmunoassay for plasma testosterone. *Z. Klin. Chem. U. Klin. Biochem.* **10**, 164-168
11. PURVIS K. and RUI H. (1988) High-affinity, calmodulin-dependent isoforms of cyclic nucleotide phosphodiesterase in rat testis. *Meth. Enzymol.* **159**, 675-685
12. SANDBERG M. A., PAWLYK B. S., CRANE W. G., SCHMIDT S. Y. and BERSON E. L. (1987) Effects of IBMX on the ERG of the isolated perfused cat eye. *Vision Res.* **27**, 1421-1430
13. SCHOEFFTER P., LUGNIER C., DEMESY-WAELEDE F. and STOCLET J. C. (1987) Role of cyclic AMP- and cyclic GMP-phosphodiesterases in the control of cyclic nucleotide levels and smooth muscle tone in rat isolate aorta. *Biochem. Pharmacol.* **36**, 3965-3972
14. SILVER P. J., HAMEL L. T., PERRONE M. H., BENTLEY R. G., BUSHOVER C. R. and EVANS D. B. (1988) Differential pharmacologic sensitivity of cyclic nucleotide phosphodiesterase isozymes isolated from cardiac muscle, arterial and airway smooth muscle. *Eur. J. Pharmacol.* **150**, 85-94
15. STEINER A. L., PARKER C. W. and KIPNIS D. M. (1972) Radioimmunoassay for cyclic nucleotides: I. Preparation of antibodies and iodinated cyclic nucleotides. *J. Biol. Chem.* **247**, 1106-1113
16. THOMPSON W. J., TERASAKI W. L., EPSTEIN P. M. and STRADA S. J. (1979) Assay of cyclic nucleotide phosphodiesterase and resolution of multiple molecular forms of the enzyme. *Adv. Cyclic Nucleotide Res.* **10**, 69-92
17. WELLS J. N. and HARDMAN J. G. (1977) Cyclic nucleotide phosphodiesterases. *Adv. Cyclic Nucleotide Res.* **8**, 119-143
18. WELLS J. N. and MILLER J. R. (1988) Methylxanthine inhibitors of phosphodiesterase. *Meth. Enzymol.* **159**, 489-496