been unsuccessful in spite of the use of various immunosuppressive agents before and after transplantation[24].
Spontaneous malignant conversion of transplanted tissues was also observed in our previous experiments[8]. It is noteworthy that no adenocarcinomas developed from untreated glandular stomach in our study, whereas there are reports that adenocarcinomas developed from untreated gastrointestinal tract of mice[11] and rats[25]. The mechanism of this phenomenon is still obscure and requires further study.

1 This work was presented at the 13th UICC Meeting in Seattle, USA, in September 1982.
2 Sugimura, T., Fujimura, S., Kogure, K., Baba, T., Saito, T., Nagao, M., Hosoi, H., Shimosato, Y., and Yokoshima, T., Gann Monogr. *13* (1972) 31.
3 Kurokawa, Y., Saito, S., and Sato, H., Gann Monogr. *17* (1975) 87.
4 Kurokawa, Y., Saito, S., and Sato, H., S. Afr. Cancer Bull. *24* (1980) 420.
5 Huh, N., Takaoka, T., and Katsuta, H., Jap. J. exp. Med. *47* (1977) 413.
6 Schryver-Kecskemet, K. De, Greider, M.H., Saks, M.K., Rieders, E.R., and McGuigan, J.E., Lab. Invest. *37* (1977) 406.
7 Kurokawa, Y., Takahashi, M., Ohno, Y., Takamura, N., and Odashima, S., Tohoku J. exp. Med. *134* (1981) 183.
8 Kurokawa, Y., Takahashi, M., Furihata, C., Ohno, Y., and Takamura, N., Gann *72* (1981) 620.
9 Smith, W.E., J. exp. Med. *85* (1947) 459.
10 Toovey, E.W., Heller, L., and Webster, D.R., Cancer Res. *11* (1951) 912.
11 Tumyan, B.G., Svetlana, N.Z., George, J.S.M., and Bertha, I.L., J. natl Cancer Inst. *52* (1974) 63.
12 Matsuyama, M., and Suzuki, H., Nagoya med. J. *20* (1975) 63.
13 Sigaran, M.F., and Con-Wong, R., Gann *70* (1979) 343.
14 Sugimura, T., and Kawachi, T., Methods in Cancer Research, vol. 7, p. 245. Academic Press, New York, London 1973.
15 Yeomans, N.D., and Trier, J.S., Devl Biol. *53* (1976) 206.
16 Nomura, T., Cancer Res. *34* (1974) 3363.
17 Dao, T.L., and Sinha, D., J. natl Cancer Inst. *49* (1972) 591.
18 Rogers, S., J. natl Cancer Inst. *15* (1955) 1675.
19 Flaks, A., and Flaks, J.O., Br. J. Cancer *22* (1968) 839.
20 Jull, J.W., Hawyluk, A., and Russel, A., J. natl Cancer Inst. *40* (1968) 687.
21 Roller, M.R., and Heidelberger, C., Int. J. Cancer *2* (1967) 509.
22 Kreider, J.W., Bartlett, B.L., and Sharkey, F.E., Cancer Res. *39* (1979) 273.
23 Shimosato, T., Kodama, T., Tamai, S., and Kameya, T., Gann *71* (1980) 402.
24 Kurokawa, Y., unpublished data, 1981.
25 Fukunishi, R., Mori, H., Kadota, A., and Yoshida, H., Trans. Jap. path. Soc. *199* (1980) in Japanese.

0014-4754/83/121404-04$1.50 + 0.20/0

Evidence that ADP-ribosylation is not necessary for luteinizing hormone stimulation of Leydig cell steroidogenesis[1]

W.H. Moger

Departments of Physiology & Biophysics and of Obstetrics & Gynecology, Dalhousie University, Halifax (Nova Scotia B3H 4H7, Canada), March 7, 1983

Summary. 3-Aminobenzamide, which inhibits ADP-ribosylation, did not inhibit luteinizing hormone's ability to stimulate androgen secretion by mouse testicular interstitial cells in vitro. 3-Aminobenzamide, but not its inactive analog 3-aminobenzoic acid, inhibited steroidogenesis stimulated by cholera toxin.

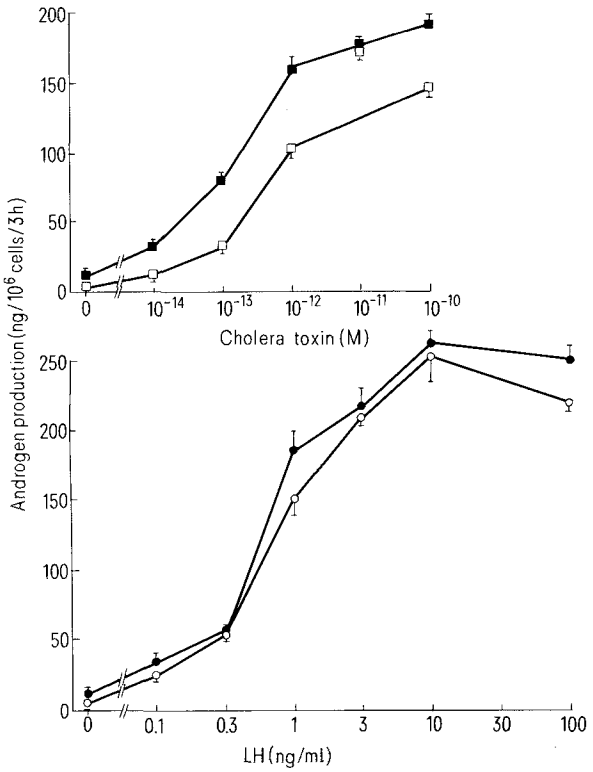
Luteinizing hormone (LH) stimulates steroidogenesis in Leydig cells by binding to cell surface receptors which increase the activity of adenylate cyclase via an ill defined interaction with the guanine nucleotide (G) regulatory subunit of the enzyme[2]. Cholera toxin also stimulates Leydig cell adenylate cyclase activity and steroidogenesis[3]; it does so by catalyzing the ADP-ribosylation of the G regulatory subunit of adenylate cyclase[4]. The possibility that ADP-ribosylation may be involved in the mechanism by which LH stimulates steroidogenesis is suggested by the recent observation that thyroid stimulating hormone (TSH), which is composed of an α-subunit identical to that of LH and a non-identical β-subunit, increases ADP-ribosyltransferase activity in thyroid membrane preparations[5–7]. TSH induced the ADP-ribosylation of the α-subunit of TSH and of a membrane protein tentatively identified as the G regulatory subunit of the adenylate cyclase complex[6–7].
The present study has utilized the competitive inhibitor of ADP-ribosyltransferase, 3-aminobenzamide[8], to test the hypothesis that ADP-ribosylation is involved in the mechanism by which LH stimulates Leydig cell steroidogenesis.
Materials and methods. Testicular interstitial cells were isolated from adult Swiss-Webster mice (High Oak Ranch, Goodwood, Ontario) as previously described[9]. 70,000 cells were suspended in 0.5 ml incubation medium (Medium 199 with Earle's salts and containing 1 mg/ml bovine serum albumin, 10 mM HEPES, 27 mM $NaHCO_3$, 27.5 mM glucose and 1 mM pyruvate). The incubation medium contained either 5 mM 3-aminobenzamide, 5 mM 3-aminobenzoic acid, or 2.5 µl of the solvent dimethylsulfoxide. Steroidogenesis was stimulated by the addition of LH (NIH-LH-B9) (0.1–100 ng/ml) or cholera toxin (10^{-14}–10^{-10} M).
The cells were incubated in an atmosphere of 95% O_2:5% CO_2 for 3 h in a shaking water bath at 33 °C. At the end of the incubation the cells were removed by centrifugation and the medium was stored at −70 °C. Unextracted 10–50 µl samples of thawed medium were added directly to the testosterone assay[10].

Effect of 3-aminobenzamide and its inactive analog, 3-aminobenzoic acid, on androgen production (ng/10^6 cells/3 h)

Inhibitor	Stimulus		
	None	Luteinizing hormone (0.3 ng/ml)	Cholera toxin (0.1 µM)
Control	3.8 ± 0.3	34.9 ± 2.1	29.3 ± 2.2
3-Aminobenzamide	3.6 ± 0.1	30.9 ± 1.7	11.7 ± 0.9*
3-Aminobenzoic acid	4.4 ± 0.4	35.6 ± 2.7	35.0 ± 1.9

Mean ± SEM of 5 replicates. * $p < 0.01$ vs control (Student's t-test).

Results and discussion. Androgen production was increased 7.7 and 9.2 times basal unstimulated production by treatment with submaximum concentrations of cholera toxin and LH, respectively, in the absence of inhibitors (table). 3-Aminobenzamide reduced by 60% cholera toxin's ability to stimulate androgen production but did not affect basal production or that stimulated by LH. The non-inhibitory analog 3-aminobenzoic acid had no effect on androgen production.



LH (lower panel) or cholera toxin (upper panel) stimulated androgen production in the presence (open symbols) or absence (closed symbols) of 5 mM 3-aminobenzamide.

The failure of 3-aminobenzamide to inhibit LH-stimulated-androgen production was not due to the LH concentration employed. With the exception of a small (12%), but significant, inhibition at 100 ng LH/ml, 3-aminobenzamide was without effect over the entire range of LH concentrations capable of augmenting androgen production (fig.).

3-Aminobenzamide at concentrations of 1–10 mM has been used by others[11–13] to investigate the role of poly (ADP-ribosyl) transferase in the control of cellular differentiation. In this present study 3-aminobenzamide (5 mM) inhibited cholera toxin's ability to stimulate androgen production, presumably by inhibiting the mono (ADP-ribosyl) transferase activity of the toxin's A subunit. The failure of 3-aminobenzamide to inhibit LH-stimulated steroidogenesis is therefore reasonable evidence that ADP-ribosylation is not part of (or at least not an obligatory part of) the mechanism by which LH stimulates steroidogenesis.

1 The technical assistance of Ms Y. Samson is gratefully acknowledged. LH was provided by Dr Leo Reichert, Jr and the NIAMDD Pituitary Hormone Distribution Program. This work was supported by grant MT-5401 from the Medical Research Council of Canada.

2 Mendelson, C., Dufau, M., and Catt, K., J. biol. Chem. *250* (1975) 8818.
3 Dufau, M.L., Horner, K.A., Hayashi, K., Tsuruhara, T., Conn, P.M., and Catt, K.J., J. biol. Chem. *253* (1978) 3721.
4 Moss, J., and Vaughan, M., A. Rev. Biochem. *48* (1979) 581.
5 Filetti, S., and Rapoport, B., J. clin. Invest. *68* (1981) 461.
6 Rapoport, B., Filetti, S., Takai, N., and Seto, P., FEBS Lett. *146* (1982) 23.
7 Vitti, P., De Wolf, M.J.S., Acquaviva, A.M., Epstein, M., and Kohn, L.D., Proc. natl Acad. Sci. USA *79* (1982) 1525.
8 Purnell, M.R., and Whish, W.J.D., Biochem. J. *185* (1980) 775.
9 Murphy, P.R., and Moger, W.H., Biol. Reprod. *27* (1982) 38.
10 Moger, W.H., J. Steril. Biochem. *13* (1980) 61.
11 Johnstone, A.P., and Williams, G.T., Nature, Lond. *300* (1982) 368.
12 Althaus, F.R., Lawrence, S.D., He, Y.-Z., Sattler, G.L., Tsukada, Y., and Pitot, H.C., Nature, Lond. *300* (1982) 366.
13 Farzaneh, F., Zalin, R., Brill, D., and Shall, S., Nature, Lond. *300* (1982) 362.

0014-4754/83/121407-02$1.50+0.20/0

Cholinomimetics produce seizures and brain damage in rats

W.A. Turski[1], S.J. Czuczwar, Z. Kleinrok and L. Turski[2]

Department of Pharmacology, Institute of Clinical Pathology, Medical School, Jaczewskiego 8, PL 20-090 Lublin (Poland), August 20, 1982

Summary. Microinjections of the cholinergic agonists, carbachol and bethanechol, either into the amygdala or into the dorsal hippocampus produced sustained limbic seizures and brain damage in rats. Systemic administration of pilocarpine in rats resulted in a sequence of convulsive disorders and widespread brain damage as well. Scopolamine prevented the development of convulsive activity and brain damage produced by cholinomimetics. These results suggest that the excessive stimulation of cholinergic muscarinic receptors can lead to limbic seizures and brain damage. It is postulated that muscarinic cholinergic mechanisms are linked to the etiology of temporal lobe epilepsy and epileptic brain damage.

The hippocampus receives via the fornix/fimbria major input from the medial septum and vertical limb of the diagonal band[3]; acetylcholine (ACh) is considered to be the neurotransmitter of this pathway[3,4]. The axons of the cell bodies of cholinergic neurons located in the medial septum and nucleus of the diagonal band enter the hippocampal formation at the level of the subfield CA 3[5], where they divide and innervate the subfields CA 1, CA 2 and CA 4[6]. A massive cholinergic innervation of the amygdala originates in the substantia innominata and the horizontal limb of the diagonal band[3]. A number of electrophysiological studies have provided evidence that the bursts elicited by ACh in