decrease in the number of olfactory sensilla on the antennae of JH-treated male *P. americana*[5,3]. In contrast, we found not only slight decrease (7.8%) in the number of s. trichoidea and s. basiconica but also a small increase (6%) in s. chaetica B on the antennae in JH-treated male *B. germanica*. Discrepancies in sensilla number for normal adult males between those reported previously[4] and this study can be attributed to insufficient sample sizes in their work (counts were based on only 5 pairs of appendages in their study)[4]. In addition, our laboratory has reported that JH-treated males do not show anomalous mating behavior and that JH treatment to female *B. germanica* results in the reduction of sensilla numbers on the antennae and maxillary and labial palps[1]. It seems that JH treatment has different effects on the numbers of sensilla in different species and sexes, and may or may not suppress the animal's behavioral repertoire in courtship. An increase in the s. chaetica B and trichoidea on the maxillary palp of treated males is of little consequence, since the maxillary and labial palps play no major role in his courtship behavior. It is reported that sensory receptors on the antennae of male *B. germanica* play a major role in the induction of courtship behavior by perceiving the female pheromone[6]. Isolated female antennae brought in contact with the male antennae only is sufficient to induce male wing-raising. Other sense organs, such as the maxillary and labial palps, did not affect the courtship response. Roth and Willis found that antennectomy alone reduced the number of males responding to females and that maxillary and labial palpectomy alone did not affect courtship. Their observations suggest that none of these organs, except the antennae, are important in the mating behavior of the male German cockroach. However, our laboratory has reported that all these organs are important in the mating behavior of the female[1].

Acknowledgment. This report is the New Jersey Agricultural Experiment Station Publication No. D-08112-12-84 supported by state funds and U. S. Hatch funds.

1 Ramaswamy, S. B., and Gupta, A. P., J. Insect Physiol. *27* (1981) 601.
2 Barth, R. H. Jr, Gen. comp. Endocr. *2* (1962) 53.
3 Schafer, R., J. exp. Zool. *199* (1977) 73.
4 Ramaswamy, S. B., and Gupta, A. P., J. Morphol. *168* (1981) 269.
5 Schafer, R., and Sanchez, T. V., J. Morphol. *9* (1976) 139.
6 Roth, L. M., and Willis, E. R., Am. Midl. Nat. *47* (1952) 66.

0014-4754/86/010057-02$1.50 + 0.20/0

Ethanol influence on insulin secretion from isolated rat islets[1]

S. P. Singh, D. G. Patel[2], A. K. Snyder and G. L. Pullen

Medical Research Service, Veterans Administration Medical Center, and Department of Medicine, Chicago Medical School, North Chicago (Illinois 60064, USA), 26 March 1985

Summary. This study was done to delineate the role of α- and β-adrenergic receptors and cyclic AMP in the mechanism of ethanol effects on insulin release from isolated islets. Rats were given an α-adrenergic blocker, phentolamine, or a β-adrenergic blocker, propranolol. In addition, ethanol 1 g/kg was given intragastrically 1 h prior to sacrifice. Glucose mediated insulin release from isolated islets was enhanced by phentolamine and decreased by propranolol. Ethanol treatment inhibited glucose-induced insulin release from isolated islets of control rats as well as those given phentolamine and/or propranolol. Insulin release from isolated islets in response to dibutyryl-cyclic AMP was attenuated by ethanol. Theophylline enhanced glucose mediated insulin release from control islets but ethanol treatment produced a significant inhibition of insulin response. The data suggest that the site of action of the deleterious effects of ethanol on insulin release from isolated islets in rat does not involve adrenergic system and cyclic AMP.
Key words. Insulin; islets; ethanol; adrenergic receptors; cyclic AMP; theophylline.

Ethanol ingestion can produce hyperglycemia and it may, in part, be due to catecholamine release[3]. The catecholamine-induced changes in glucose homeostasis are the product of several factors, including an enhancement of hepatic glycogenolysis, gluconeogenesis, inhibition of peripheral glucose utilization, and indirectly via pancreatic hormone secretions[4,5]. The relative contribution of each of these factors for the total hyperglycemic response to ethanol-induced catecholamine release is unknown. Previously, studies from our laboratory and of others have shown that ethanol inhibits insulin secretory response to glucose and tolbutamide in intact rat and in isolated rat islets[6–8]. The underlying mechanism for these observations is not clear, although Malaisse et al.[9] have suggested that ethanol can interfere with microtubular functions of β-cells. Since ethanol stimulates catecholamine secretion[10] and insulin release is inhibited by the α-adrenergic system but enhanced by the β-adrenergic system[5], it is possible that ethanol alters insulin secretion, via the α-and/or β-adrenergic system. Intracellular regulatory mechanisms in insulin secretion involve cyclic AMP; and inhibition of insulin secretion by ethanol may be mediated by the catecholamines effect on cyclic AMP. Secondly, Kuo et al.[11] reported that ethanol exerts an inverse dose-dependent influence on adenyl cyclase activity in the homogenate of rat islets. It is likely, therefore, that the underlying mechanism of ethanol-induced inhibition of insulin response to glucose involves catecholamines and islet cyclic AMP.

The present study was therefore done to determine the role of α- and β-adrenergic receptors as well as of cyclic-AMP in ethanol effect on glucose-induced insulin release. The data show that inhibitory effect of ethanol on insulin secretion from isolated rat islets is not mediated by perturbations in adrenergic receptors activity or cyclic AMP.

Materials and methods. Male Sprague-Dawley rats, weighing 400 ± 25 g, were housed individually in conditions of controlled temperature, humidity and light cycle, and were given Purina chow and water ad libitum. One rat of each pair was given ethanol (1 g/kg) intragastrically 1 h prior to sacrifice, whereas the other animal received saline. The dose and time of ethanol administration prior to sacrifice were selected in view of our previous studies to determine ethanol effects on insulin secretion in isolated rat islets[8]. Based essentially upon experiments in rats by Luyckx and Lefebvre[12], both rats were given either propranolol (2 mg/kg) or phentolamine (20 mg/kg) or both i.p. twice, 3 and 1 h prior to the sacrifice, to block α- and/or β-adrenergic receptors.

Isolated islet studies. The method of isolation of rat islets has previously been described[13]. Briefly, animals were anesthetized with sodium nembutal i.p. (50 mg/kg b.wt), blood was taken by cardiac puncture for ethanol estimation, and the islets isolated from the pancreas using collagenase digestion. Approximately 100 well preserved islets were harvested from the individual pancreas. Islets were divided into batches of 10 each and prein-

Ethanol effect on insulin release from isolated islets of animals treated with α and β adrenergig receptor blocking drugs

Group	Insulin release (μU/10 islets/30 min) Saline	Phentolamine	Propranolol	Phentolamine + propranolol
Control	864 ± 16	1283 + 23	539 ± 15	935 ± 17
Ethanol	642 + 25	690 ± 16	132 ± 4	258 ± 8
p		< 0.001	< 0.001	< 0.001

Animals were given i.p. phentolamine 20 mg/kg or propranolol 2 mg/kg twice, 3 and 1 h, prior to sacrifice. Controls received saline. 1 h prior to sacrifice, ethanol 1 g/kg or saline was given by intragastric route. Isolated islets were incubated with 16.7 mM glucose to determine insulin release as given in the text. Results are shown as mean ± SEM, n = 24.

cubated in 1.0 ml modified Krebs Ringer bicarbonate (KRB) pH 7.4, containing 1.67 mM glucose and 0.4% w/v bovine serum albumin under 95% 0_2–5% CO_2 mixture at 37 °C in a Dubnoff shaker. After preincubation, the media were discarded, the islets washed twice with saline and then incubated in media containing insulin stimulatory concentration of glucose and/or other drugs as indicated under the results. Immediately after addition of media to the islets, an aliquot was taken for baseline (t_0) insulin level and thereafter an aliquot was taken after 30 min of incubation (t_{30}) for insulin release response to glucose. The aliquots were stored at −20 °C until assayed for immunoreactive insulin (IRI).

Dibutyryl-cyclic AMP and theophylline studies. In the first set of experiments, islets of rats given ethanol 1 g/kg or saline intragastrically 1 h prior to sacrifice were incubated in modified KRB enriched with 1×10^{-3} M dibutyryl-cyclic AMP with or without glucose to stimulate IRI release. In the second set of experiments, islets were incubated in modified KRB containing 10 mM theophylline with or without 16.7 mM glucose. After 30 min incubation, insulin release from islets was determined as described above.

Analytical technique. IRI levels were determined by the double antibody radioimmunoassay method of Morgan and Lazarow as modified by Blanks and Gerritsen[14]. Rat insulin kindly supplied by the Eli Lilly Company, Indianapolis, IN, was used as the standard. Net insulin release from each group of islets was calculated as the difference in insulin concentration in the media between t_{30} and t_0 and expressed as μU/10 islets/30 min. Blood ethanol levels were estimated by an enzymatic method[15]. The data were analyzed by analysis of variance (ANOVA) and the results are represented as arithmetic mean ± standard error (mean ± SEM).

Results. Ethanol effect on insulin release from isolated islets of rats pretreated with α and β adrenergic blockers. The table shows mean ± SEM, N = 24, (batches of 10 islets each), values of glucose mediated insulin release from isolated islets of rats given ethanol and pretreated with α and/or β adrenergic receptor blocking drugs. The data from control rats which were not given α or β receptor blockers but received saline or ethanol is given as well. In control rats, the α-adrenergic blocker increased IRI secretory response to glucose by 48% while the β-adrenergic blocker resulted in 38% decrease in IRI release as compared to corresponding values observed in rats which did not receive α or β adrenergic blockers. Ethanol administration significantly inhibited insulin response to glucose in all groups of animals; p-values are shown in the table. Blood ethanol levels were nearly equal in all groups of animals. Therefore, mean ± SEM level calculated as combined values was 95 ± 2.1 mg% (20.6 ± 4.6 mM).

Ethanol effect on insulin response of isolated islets in the presence of dibutyryl-cyclic AMP and theophylline. To investigate whether ethanol influence on IRI secretion affected cyclic AMP in the islets, two sets of experiments were done. First, islets from ethanol or saline treated rats were incubated with either 10 mM dibutyryl-cyclic AMP or 11.2 mM glucose or both. The concentration of glucose as insulin secretagogue used was submaximal since other investigators[16] and our preliminary experiments (glucose concentration used being 1.67, 5.3, 11.2 and 16.7 mM) have indicated it to be appropriate to investigate effects of dibutyryl-cyclic AMP on insulin release. Ethanol significantly diminished insulin secretory response to dibutyryl-cyclic AMP and to 11.2 mM glucose as compared to control values. Actual mean ± SEM values of insulin release (μU/10 islets/30 min) from islets of control and ethanol-fed rats were as follows: 494 ± 40 vs 296 ± 28 in the presence of 11.2 mM glucose; 498 ± 5 vs 303 in the presence of 10 mM dibutyryl-cyclic AMP; and 1064 ± 57 vs 579 ± 43 in the presence of combined stimulus of 11.2 mM glucose and 10 mM dibutyryl-cyclic AMP. p-Values of all comparisons were < 0.001, n = 17–19.

In a second set of experiments, the addition of 10 mM theophylline to the incubation media of isolated rat islets of control rats enhanced insulin response to 16.7 mM glucose; actual values being 1234 ± 49 vs 751 ± 21 μU/10 islets/30 min for glucose + theophylline and glucose alone respectively; n = 17. Ethanol treatment produced 34% inhibition (p < 0.0001) of IRI release from islets exposed to glucose + theophylline; this was nearly equal to the inhibitory effect of ethanol on IRI release from islets exposed to glucose alone. Theophylline in the absence of glucose was inadequate as insulin secretagogue as insulin release was minimal above the baseline values (54 ± 11 μU/10 islets/30 min).

Discussion. The inhibitory effect of catecholamines on glucose-induced insulin secretion has been demonstrated in man and other species[5,17] as well as in studies of pancreatic slices[18], in isolated islets[19] and in isolated perfused rat pancreas[20]. Phentolamine treatment, besides producing blockade of α receptors, also unmasks the β receptor action and thereby enhances insulin release. Converse effects are observed with propranolol treatment as β receptors are blocked and α receptors are unmasked, with the result that insulin secretion is decreased. The present data are in conformity with these observations, as insulin secretory response to glucose was increased by phentolamine but decreased in islets of propranolol-treated animals. It should be noted that phentolamine and propranolol were given in vivo, whereas insulin release was determined in isolated islets in vitro. The observed changes in insulin secretion in response to adrenergic blocking by phentolamine and propranolol remain effective despite the washing and preincubation of the islets. Cohen et al.[10] reported that a single dose of ethanol g/kg) given to rats 90 min before sacrifice exerted a direct effect on synthesis and release of catecholamine from the adrenal gland. In the present study, ethanol attenuated insulin secretion despite α and β receptor blockade. This suggests that ethanol influence on glucose mediated insulin release does not involve the participation of the adrenergic system.

The precise role of cyclic AMP in insulin secretion is unclear but it has been shown to be a positive modulator of insulin secretion[16,21,22]. Since ethanol affects adenyl cyclase activity in rat islet[11], it is possible that ethanol inhibition of insulin secretion might involve a decrease in cyclic AMP level. Unfortunately, islet cyclic AMP levels in the islets were not measured and this possibility cannot be excluded by the present study. However, the data show that ethanol inhibits insulin release despite the presence of dibutyryl-cyclic AMP or theophylline in the incubation media. Both theophylline and dibutyryl-cyclic AMP are known to increase intracellular level of cyclic AMP. Thus, irrespective of ethanol effect on adenyl cyclase activity, the inhibitory influence of ethanol on insulin secretion involves steps beyond the generation of cyclic AMP. These observations are in conformity with a previous in vivo study, wherein simultaneous infusion of ethanol and cyclic AMP into pancreatic artery inhibited cyclic AMP induced insulin release in dog[23].

As reviewed by Malaisse and coworkers[22], the events within the β-cell leading to insulin secretion in response to glucose involve

three major steps: a) the recognition of glucose as a stimulus and glucose metabolism to generate cofactors such as ATP and NADPH; b) alterations of ionic fluxes across the plasma membrane and membranes of intracellular organelle, in particular resulting in cytosolic accumulation of Ca^{++}; and c) contractility of microtubular-microfilamentous system to translocate insulin granules and their eventual release by exocytosis. The present study suggests that apparently adrenergic receptors and cyclic AMP are not involved in the mechanism by which ethanol inhibits insulin secretion. However, based upon well documented studies on the metabolic effects of ethanol [24], it is conceivable that ethanol may interfere with insulin release by its action at other sites of the sequence of events involved in insulin release.

1 Supported by the U.S. Veterans Administration.
2 Present address: Department of Pharmacology, Meharry Medical College, 1005–18th Avenue North, Nashville, Tennessee 37208, USA.

3 Ritchie, J.M., in: The Pharmacological Basis of Therapeutics p. 376. Eds Goodman, A.G., Goodman, L.S. and Gilman, A., MacMillan Publishing Co., Inc., New York 1970.
4 Sloan, I., Sawh, P., and Bihler, I., Molec. cell. Endocr. *10* (1978) 3.
5 Unger, R.H., Dobbs, R.E., and Orci, L., A. Rev. Physiol. *40* (1978) 307.
6 Shah, J.H., Wongsurawat, N., and Aran, P.P., Diabetes *26* (1977) 271.
7 Singh, S.P., and Patel, D.G., Metabolism *25* (1976) 239.
8 Singh, S.P., Patel, D.G., and Snyder, A.K., Acta endocr. *93* (1980) 61.
9 Malaisse, W.J., Malaisse-Lagae, F., Walker, M.O., and Lacy, P.E., Diabetes *20* (1971) 257.
10 Cohen, L.B., Sellers, E.M., Sellers, E.A., and Flattery, D.W., J. Pharmac. exp. Ther. *212* (1980) 425.
11 Kuo, W.N., Modgins, D.S., and Kuo, J.F., J. biol. Chem. *248* (1973) 2705.
12 Luyckx, M.D., and Lefebvre, P.J., Diabetes *23* (1974) 81.
13 Patel, D.G., and Singh, S.P., Metabolism *28* (1979) 85.
14 Blanks, M.C., and Gerritsen, G.C., Proc. Soc. exp. Biol. Med. *146* (1974) 448.
15 Bonnichsen, R., and Theorell, H., Scand. J. clin Lab. Invest. *3* (1951) 58.
16 Wollheim, C.G., and Sharp, G.W.G., Physiol. Rev. *61* (1981) 914.
17 Malaisse, W.J., Brisson, B., and Malaisse-Lagae, F., J. Lab. clin. Med. *76* (1970) 895.
18 Coore, H.G., and Randle, P.J., Biochem. J. *93* (1964) 66.
19 Malaisse, W., Malaisse-Lagae, F., Wright, P.H., and Ashmore, J., Endocrinology *80* (1967) 975.
20 Loubatieres, A., Mariani, M.A., and Chapal, J., Diabetologia *6* (1970) 533.
21 Fain, J.N., Metabolism *33* (1984) 672.
22 Malaisse, W.J., Sener, A., and Malaisse-Lagae, F., Molec. cell. Biochem. *37* (1981) 157.
23 Colwell, A.R., Feinzimer, M., Cooper, D., and Zuckerman, L., Diabetes *22* (1973) 854.
24 Hawkins, R.D., and Kalant, H., Pharmac. Rev. *24* (1972) 67.

0014-4754/86/010058-03$1.50 + 0.20/0

Comparison of the time courses of luteinizing hormone (LH) secretion rates during continuous stimulation by LH-releasing hormone (LH–RH) in vivo and in vitro

J. de Koning, A.M.I. Tijssen, J.A.M.J. van Dieten, T.R. Koiter*, G.A. Schuiling* and G.P. van Rees

*Department of Pharmacology of the University of Leiden, P.O. Box 9503, 2300 RA Leiden (The Netherlands), and *Department of Obstetrics and Gynecology, Academic Hospital, Oostersingel 59, 9713 EZ Groningen (The Netherlands), 20 May 1985*

Summary. The patterns of LH secretion during constant stimulation of the pituitary glands of estradiol-treated ovariectomized rats with a maximally stimulating amount of LH–RH in vivo and in vitro correspond with each other qualitatively and quantitatively. In vitro the changes with time of the LH secretion rate are somewhat retarded, especially the occurrence of desensitization.
Key words. Pituitary gland; luteinizing hormone (LH); luteinizing hormone-releasing hormone (LH–RH); LH–RH releasing activity in vivo vs in vitro.

Biochemical studies of the processes which are activated by stimulation of the pituitary gland with LH–RH are mostly performed in vitro[1,2]. The ultimate result of the activation of those processes, the secretion of LH, has a very characteristic pattern which, however, varies according to the experimental design (hemi-pituitary glands, dissociated pituitary cells) used[2–6], and depends on the endocrine state (e.g. intact, gonadectomized, hormone-treated) of the animal from which the pituitary glands have been collected[7–10]. From the cited literature it also appeared that the patterns of LH secretion in vivo are often different from those observed in vitro. Consequently, the close relationship between the biochemical processes studied in vitro and the pattern of LH secretion in vivo remains unclear. In the present study, a first step was made towards clarifying that relationship by investigating the kinetics of LH secretion rates during continuous stimulation with LH–RH both in vitro, by incubating hemi-pituitary glands with constant amounts of LH–RH, and in vivo, by infusing LH–RH at a constant rate.

Materials and methods. Animals. Adult female rats from the Wistar-derived colony kept in the Department of Pharmacology in Leiden were used. They were allowed free access to food and water in an animal room illuminated from 05.00 to 19.00 h, at a constant temperature of 22 °C. The animals were ovariectomized (OVX) irrespective of the stage of the ovarian cycle and used 14 days afterwards; at that time they weighed about 200 g. For methodological reasons (increased responsiveness of the pituitary glands to LH–RH) the animals were s.c. injected with estradiol-17β-benzoate (OB; Organon, Oss, The Netherlands; 7 µg in 0.2 ml of arachis oil) 24 h before the start of the experiment.

Infusions. A maximally active amount of LH–RH[11] (Beckman, Geneva, Switzerland) was infused at a constant rate of 1000 ng/h via a cannula inserted into the right jugular vein. Blood samples were taken through a cannula inserted into the right carotid artery. One hour before the start of the infusion and, if relevant 4 h later, the animals received (an) i.p. injection(s) of sodium phenobarbitone (80 mg/kg b.wt) in order to suppress endogenous LH–RH release.

Incubations. The animals were killed by decapitation. Each anterior pituitary gland was halved and placed in an incubation flask containing medium TC 199 (Boehringer, Mannheim, W. Germany). They were preincubated in the same medium until 20 min following decapitation. Then the medium was replaced by 1 ml medium with or without a maximally active concentration of LH–RH[7,8] (1000 ng/ml) and a 4-h incubation followed. The incubations were carried out at 37 °C under continuous shaking in an atmosphere of O_2:CO_2 (95%:5%).

Assay of LH. The assay used was essentially the same as that described by Welschen et al.[12]. Specific anti-ovine LH was a generous gift from Drs J. Dullaart and J.Th.J. Uilenbroek