Life Sciences, Vol. 52, pp. 687-694 Pergamon Press
Printed in the USA

SUPPRESSIVE EFFECT OF GABA ON INSULIN SECRETION FROM THE PANCREATIC BETA-CELLS IN THE RAT

Xiao-Hong Gu*, Takeshi Kurose, Seika Kato,
Kazuhiro Masuda, Kinsuke Tsuda, Hitoshi Ishida, and Yutaka Seino

Department of Metabolism and Clinical Nutrition, Kyoto University Faculty of Medicine, Kyoto, Japan, *2nd Department of Internal Medicine, East Suburbs First Workers Hospital, Xian, Shaanxi, P.R. China

(Received in final form December 4, 1992)

Summary

In order to investigate a possible role of GABA in the regulation of insulin secretion, we have studied the effect of GABA on insulin secretion from the isolated perfused rat pancreas in vitro and on the changes in the cytoplasmic Ca^{2+} of Beta-cells from the isolated rat islets. When glucose is present, GABA caused a dose dependent inhibition of the first phase of arginine-induced insulin secretion during the range of 10-1000 μM, but GABA did not affect arginine-induced insulin secretion in the absence of glucose. GABA inhibited not only the first phase but also the second phase of glucose-induced insulin secretion. A $GABA_B$-receptor agonist, baclofen, also inhibited both phases of insulin secretion induced by 16.7 mM glucose. Furthermore, GABA inhibited the rise in cytoplasmic Ca^{2+} of Beta-cells in response to 16.7 mM glucose. These studies indicate that GABA decreases Beta cell secretory activity mainly in response to glucose. These inhibitory effects of GABA on insulin secretion may be mediated through $GABA_B$-receptor and the inhibition of the rise in cytoplasmic Ca^{2+}.

γ-aminobutyric acid (GABA) has been reported to be one of the most important inhibitory neurotransmitters in the mammalian central nervous system (1). GABA and its synthesizing enzyme glutamate decarboxylase (GAD) have also been found in the endocrine pancreas (2). Recently, evidences for a role of GABA as a paracrine modulator in the pancreatic islets have been reported (3,4). The effects of GABA on pancreatic Alpha- and Delta-cells are believed to be mediated through $GABA_A$-receptors.

However, the effect of GABA on insulin release has not been well characterized, since differences in experimental design or species of animals may have yielded contradictory results. In our present study we have investigated the effects of GABA and baclofen on insulin secretion from the isolated perfused rat pancreas. Furthermore, in order to elucidate the mechanism of regulation of insulin secretion by GABA, we studied the changes in cytoplasmic Ca^{2+} in Beta-cells loaded with the Ca indicator, fura-2 using isolated rat islets.

Correspondent: Yutaka Seino, MD, Department of Metabolism and Clinical Nutrition, Kyoto University Faculty of Medicine, 54 Shogoin Kawahara-cho, Sakyo-ku, Kyoto 606, Japan
Phone: 81-75-751-3560, Fax: 81-75-771-6601

0024-3205/93 $6.00 + .00

Materials and Methods

Male Wistar rats weighing 200-250 g were housed for at least a weak in a temperature- and light-conditioned room. After an overnight fast, anesthesia was introduced by intraperitoneal administration of 6 mg/100 g body weight pentobarbital sodium. The isolated and perfused rat pancreas was prepared by the method described previously (5). All perfusions were performed with Krebs-Ringer bicarbonate buffer containing 0.25 % bovine serum albumin (fraction V, Sigma Chemical, St. Louis, MO) and 4.6 % dextran (mean mol. wt. 70,000, Pharmacia, Sweden). The flow rate through the pancreas was usually 1.9 ml/min. Each 1-min effluent from the portal vein was collected into chilled tubes containing 1,000 U of trasylol (Bayer, Leverkusen, Germany), frozen immediately, and stored at -20 °C until assayed. The first series of experiments assessed the ability of GABA (Sigma Chemical) to inhibit insulin secretion induced by arginine infusion. L-arginine hydrochloride (Sigma Chemical), to provide a final concentration of 10 mM, was introduced over 20 min through a side arm pump. Glucose was maintained at a final concentration of 5.5 mM. 10, 100, 1000 μM GABA were administered over whole experimental periods, including the equilibration period of 20 min. The second series of experiments were performed to assess the effect of GABA and baclofen (Sigma Chemical), a $GABA_B$-receptor agonist, on insulin secretion induced by a high concentration of glucose infusion. 100 μM GABA or 300 μM baclofen were maintained over whole experimental periods. Five min after the start of sampling, glucose concentration was changed from 5.5 mM to 16.7 mM, and was maintained for 20 min in the GABA experiment and for 10 min in the baclofen experiment. The third series of experiments were performed to assess the effect of GABA on changes in $[Ca^{2+}]i$ levels of Beta-cells induced by a high concentration of glucose. Islets of Langerhans were isolated from male Wistar rats weighing 180-200 g by collagenase digestion (6). Single islet cells were preparated by shaking in a Ca^{2+}-deficient medium, washed twice, and the islet cells adhered on the cover slips either in a single cell or small cluster configuration. The islet cell viability assessed by trypan blue exclusions was greater than 95%. The islet cells were then loaded with 1.0 μM fura-2 acetoxymethylester (Molecular Probes, Eugene, OR) for 30 min in Krebs-Ringer-Bicarbonate buffer (KRBH) containing 129.4 mM NaCl, 5.2 mM KCl, 2.7 mM $CaCl_2$, 1.3 mM KH_2PO_4, 1.3 mM $MgSO_4$, 24.8 mM $NaHCO_3$, 10 mM HEPES, 3.3 mM glucose, and 0.2% BSA, pH 7.4 at 37°C, and placed in the square well (500 μl in bed volume) which was mounted on the stage of the microscope. The cells were then superfused with KRBH containing 3.3 mM glucose for 10 min and successively challenged with 16.7 mM glucose with or without 1000 μM GABA at a flow rate of 2 ml/min for 10 min. The superfusion was performed at 37°C by heating the stage of microscope and the medium. Dual-excitation wavelength measurement (340 and 360 nm) was permitted by an automatic device allowing two wavelengths to be alternatively selected using microcomputer-driven shutters. The islet cells were viewed with a X 20 fluorescence objective (Nikon, Tokyo, Japan). Fluorescence emission at 510 nm was monitored with a silicon-intensified target camera (C2400-08H, Hamamatsu Photonics, Hamamatsu, Japan), and the ratio calculation was digitized every 20 seconds by a computerized image processor (Argus-100/CA, Hamamatsu Photonics). The experiments were always performed on individual cells more than 10 μm in diameter and not in contact with other cells, because it has been reported that the non-insulin producing cells are characterized by their smaller size (7). In vitro calibration was made in a range of pCa 9-5, and the 340/360 fluorescence ratio was converted into calibrated values of $[Ca^{2+}]i$ (8).

The concentration of insulin in the perfusate was measured by a specific radioimmunoassay as previously described (9). The sum of first phase insulin release was calculated as the sum of released insulin above the basal level of insulin secretion during initial 10 min after the secretagogues were administered. The total increment above the basal level of insulin secretion is the sum of the difference between each value during the whole period of administration of secretagogues and basal value just before administration. $\Delta[Ca^{2+}]i$ was calculated as the difference between the peak value during the administration of 16.7 mM glucose and the prestimulatory value. Statistical comparisons of means among two groups were made using student's *t* test. Comparisons between means of more than three groups were made analysis of variance followed by Duncan's multiple range test (10), if null hypothesis were rejected by the former. Differences were accepted as significant at $p<0.05$.

Results

Effects of various concentration of GABA on arginine-induced insulin secretion.

Fig. 1 shows the mean hormone levels of 5 perfusion experiments. In the presence of 5.5 mM glucose the first phase of arginine-induced insulin secretion was inhibited by 100 μM or 1000 μM GABA ($p<0.05$, Table 1). The inhibition seemed to be dose related. The second phase, however, was not inhibited by any dose of GABA.

Effects of GABA and baclofen on glucose-induced insulin secretion.

As shown in Fig. 2, 100 μM GABA inhibited both phases of insulin secretion induced by glucose. The total increment above basal level of insulin secretion during 16.7 mM glucose infusion was 21.5±4.7 ng/20 min, significantly lower than the value, 57.5±14.3 ng/20 min, in the control group ($p<0.05$, Table 2). The effects of baclofen on glucose-induced insulin secretion are shown in Fig. 3. Baclofen also inhibited glucose-induced insulin secretion, and the increment above basal level of insulin secretion in the group treated by baclofen was significantly lower than in the control group ($p<0.05$).

Effects of GABA on $[Ca^{2+}]i$ levels of Beta-cells loaded with fura-2.

Fig. 4 shows representative traces of the $[Ca^{2+}]i$ rise in response to 16.7 mM glucose in the presence or absence of 1000 μM GABA in Beta-cells. The prestimulatory cytoplasmic Ca^{2+} levels in the control group, 44.8±1.7 nM, was similar with the GABA group. Glucose caused an increase in $[Ca^{2+}]i$ with a small initial rise followed by a plateau phase in the control group. In the presence of GABA the rise of $[Ca^{2+}]i$ was observed in response to glucose, however, the estimated increment of $[Ca^{2+}]i$ above the basal level in the GABA-treated group was significantly lower than in the control group ($p<0.001$, Table 3).

Discussion

In the present study, GABA caused a dose-dependent inhibition of the first phase of arginine-induced insulin release. In a previous report GABA inhibited steady-state insulin secretion induced by 10 mM arginine in the presence of 5.5 mM glucose from the isolated perfused dog pancreas (11). Since the mechanisms involved in the insulin secretion induced by arginine still remain unclear, it may be difficult to identify the exact site through which GABA modulate arginine-induced insulin release. Arginine, however, stimulates insulin secretion in the absence of glucose, and causes monophasic release without acute spike (within 10 min after secretagogues are administered) (12). By contrast, in the presence of basal level of glucose arginine can stimulate insulin secretion in a biphasic manner as is observed in insulin secretion in response to a high glucose concentration. Accordingly inhibition of the first phase of insulin release during arginine infusion induced by GABA may be caused by some interaction with the secretory mechanism by glucose. Furthermore, since we have observed no apparent effect of GABA on arginine-induced monophasic insulin secretion in the absence of glucose (data not shown), it seems likely that GABA acts on glucose-induced insulin secretory pathway.

In the second line of our study, we have investigated the effect of GABA on glucose-induced insulin release. GABA inhibited both phases of insulin release induced by 16.7 mM glucose. Taniguchi et al. (13) have observed, however, that GABA had no effect on glucose-stimulated insulin secretion using the isolated rat islets. By contrast in the incubated rabbit pancreas pieces GABA augmented glucose-induced insulin secretion with the peak effective concentration of 10^{-5} M (14). The failure of other investigators to find the suppressive effect on insulin secretion induced by glucose is likely to be the use of different experimental protocol. We have administered GABA throughout the perfusion period including the prestimulatory period, whereas previous investigators have used GABA more acutely and studied the effect on the stimulated insulin

TABLE 1

Effect of GABA on arginine-induced insulin secretion

	n	FIRST PHASE (ng)	TOTAL (ng)
CONTROL	5	63.7±9.3	86.3±11.3
GABA 10 μM	5	49.6±13.0	71.1±16.8
100 μM	5	39.7±4.3	60.8±6.6
1000 μM	5	21.6±3.9*#	38.6±8.4*

*P<0.05 vs. CONTROL. #P<0.05 vs. GABA 10 μM.

TABLE 2

Effect of GABA on glucose-induced insulin secretion

	n	FIRST PHASE (ng)	TOTAL (ng)
CONTROL	5	20.1±4.7	57.5±14.3
GABA 100 μM	5	7.8±1.7*	21.5±4.7*
CONTROL	5	20.3±3.6	____
BACLOFEN 300μM	5	10.8±1.5*	____

*P<0.05 vs. CONTROL.

TABLE 3

Effect of GABA on changes in $[Ca^{2+}]i$ levels of Beta-cells loaded with fura-2 in response to 16.7 mM

	n	$\Delta[Ca^{2+}]i$ (nM)
CONTROL	39	58.9±3.7
GABA 1000 μM	24	28.4±5.6*

*P<0.001 vs. CONTROL. Means (±SE) of indicated numbers of observations are shown.

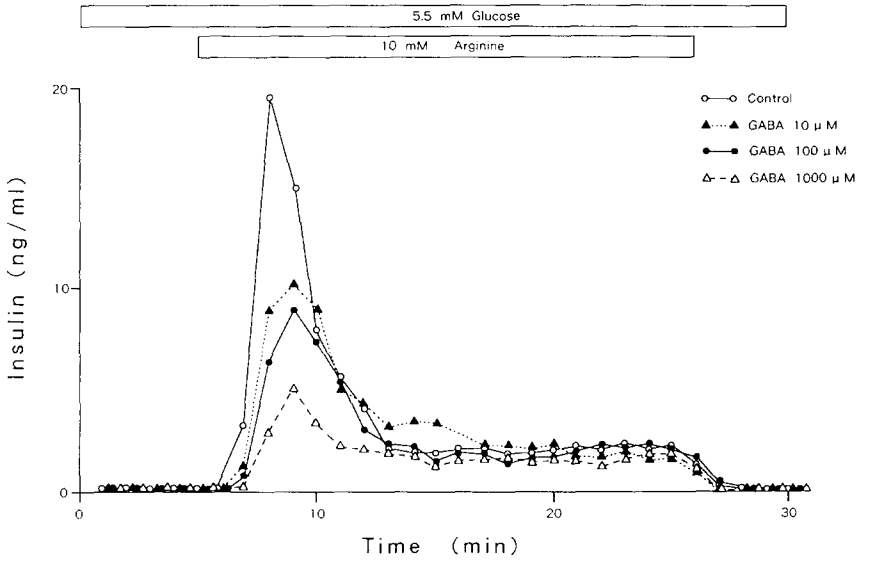

Fig. 1

Insulin secretion in response to 10 mM arginine during perfusion of the isolated and perfused rat pancreas with varying doses of GABA. The perfusate contained 5.5 mM glucose. Means (±SE) of five individual experiments are shown.

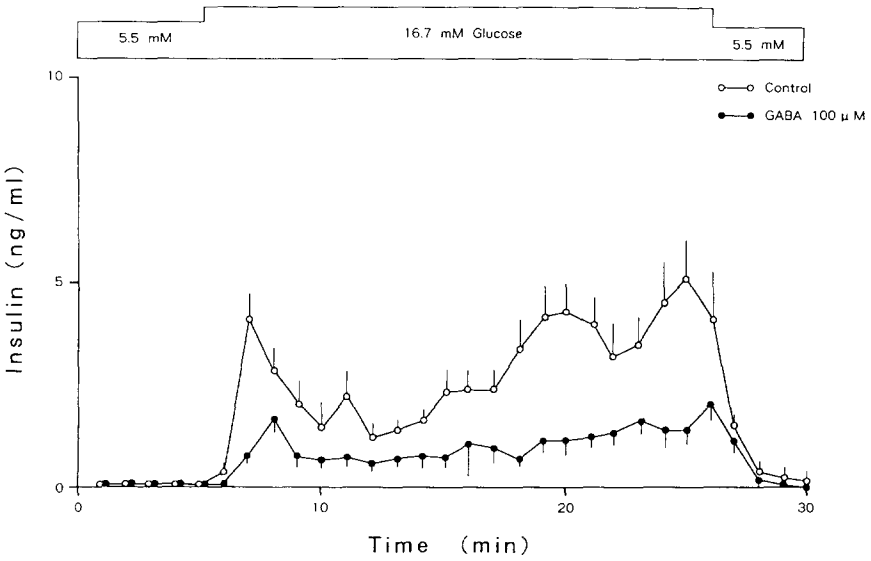

Fig. 2

Insulin secretion in response to 16.7 mM glucose during perfusion of the isolated and perfused rat pancreas with or without 100 μM GABA. Means (±SE) of five individual experiments are shown.

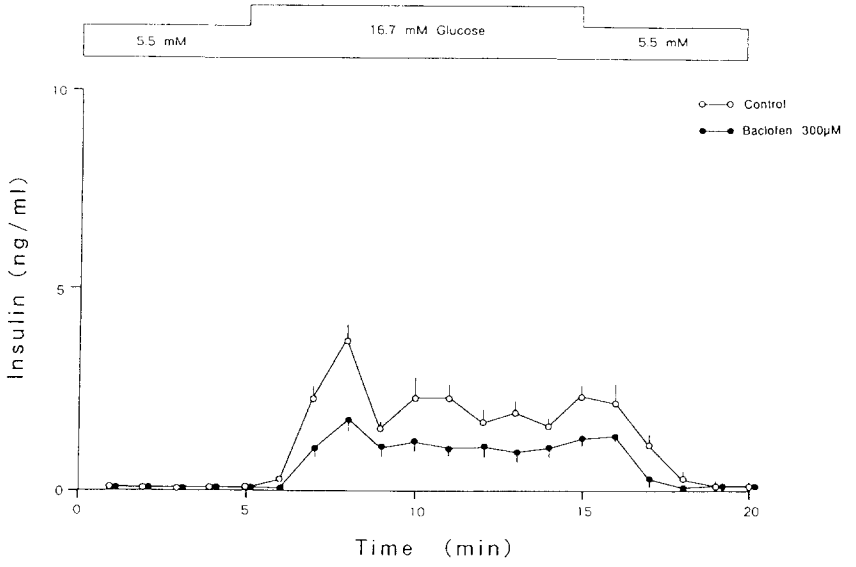

Fig. 3

Mean (±SE) levels of insulin in response to 16.7 mM glucose during perfusion of the isolated and perfused rat pancreas with or without 300 µM baclofen. Means of five individual experiments are shown.

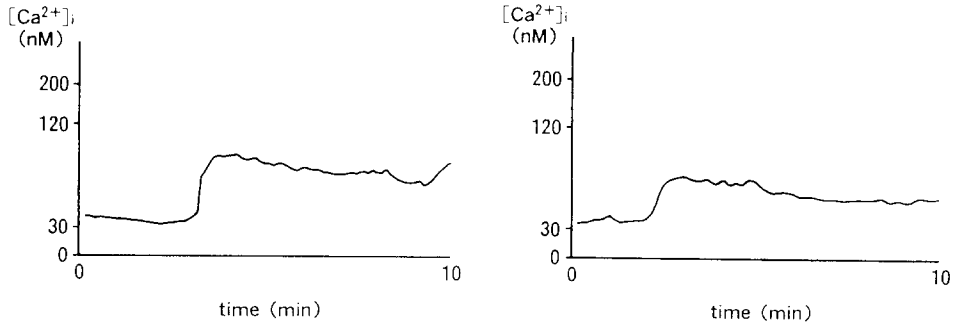

Fig. 4

Representative traces of the $[Ca^{2+}]i$ rise in response to 16.7 mM glucose in the hpresence or absence of 1000 µM GABA in Beta-cells. Application of the high concentration of glucose (16.7 mM) was started 1 min after the beginning of monitoring of $[Ca^{2+}]i$ (left). 1000 µM GABA was applied with 16.7 mM glucose 1 min after the start of monitoring of $[Ca^{2+}]i$ (right).

release. Although there remains a lack of convincing evidence, GABA has been strongly suggested to be released from Beta-cells (15). Therefore, GABA might be involved in the autocrine regulatory system in Beta-cells, and this seems to be the reason why the relatively high concentration of GABA was needed to inhibit the glucose-stimulated insulin release from Beta-cells. In the present study we have administered 10-1000 μM GABA at a rate of 1.9 ml/min,which corresponds to exposing the pancreas to approximately 5-500 nmol GABA/g wet wt/min. Gerber et al. (14) has reported that the rat pancreas contains approximately 40 nmol GABA/g wet wt. It is, accordingly, probable that the Beta-cell may be exposed to these high concentrations of GABA in vivo, since most of the pancreatic GABA is localized in the islets. It seems likely that GABA is present in the pancreas at sufficient concentrations to inhibit insulin secretion under physiologic conditions. Furthermore in the present study, since baclofen also inhibited the glucose-induced insulin release, GABA may act as a "long term" autocrine regulator of insulin release through a $GABA_B$-receptor mediated mechanism. In addition, it has been reported in the central nervous system that the presynaptic $GABA_B$-receptor might be involved in the autoreceptor mechanism as well as $GABA_A$-receptor (16).

The mechanism of suppressive effect of GABA on glucose-induced insulin release is still unclear. GABA, however, acts on the control mechanism of cytoplasmic Ca^{2+} levels of the Beta-cell, since in our present study GABA significantly affected cytoplasmic Ca^{2+} levels in a single cell or small cluster of Beta-cells in response to glucose challenge. The signaling pathway that follows the activation of $GABA_B$-receptor has been reported in relation with the G-protein mediated inhibition of the voltage dependent calcium channels (VDCC) or activation of K-channels in the CNS (17). It has been reported that arginine depolarizes the membrane potential as a cationic molecule, thus arginine stimulates Ca influx through VDCC in the pancreatic Beta-cells (18). However, since we could not observe any apparent effect of GABA on arginine-induced insulin release in the absence of glucose, it seems unlikely that GABA acts on VDCC in the inhibition of insulin release. On the other hand, the inhibitory effect of GABA on glucose-induced insulin secretion may be involved in the regulation of K-channels through G-protein coupled $GABA_B$-receptors, since the efflux of K^+ drives the membrane potential down (17). Although we did not observe the activity of K-channels, or VDCC of Beta-cells, the stimulation of K^+ efflux in response to activation of $GABA_B$-receptor might predominantly affect changes in cytoplasmic Ca^{2+} levels in response to glucose.

Acknowledgements

The study was supported in part by Grants-in-Aid for Scientific Research from the Ministry of Education, Science, and Culture of Japan and a grant for diabetes research from Otsuka Pharmaceutical Lo., Ltd. We thank Ms. Hiroko Tachikawa, Ms. Toshiko Sokai, and Mr. Tsutomu Tanimura for their skilled technical assistance.

References

1. R.L. SORENSON, D.G. GARRY, and T.C. BRELJE, Diabetes 40 1365-1374 (1991)
2. Y. OKADA, H. TANIGUCHI, and C. SHIMADA, Science 194 620-622 (1976)
3. P. RORSMAN, PER-OLOF BERGGREN, K. BOKVIST, H. ERICSON, H. MÖHLER, CLAES-GÖRAN ÖSTENSON, and P.A. SMITH, Nature 341 233-236 (1989)
4. M.S.ROBBINS, L.H. GROUSE, R.L. SORENSON, and R.P. ELDE, Diabetes 30 168-171 (1981)
5. S. SEINO, H. SAKURAI, Y. SEINO, K. TSUDA, K. TANIGAWA, H. KUZUYA, Y. GOTO, and H. IMURA, Diabetes 29 323-325 (1980)
6. M. GOTOH, T. MAKI, T.KIYOIZUMI, S. SATOMI, and A.P. MONACO, Transplantation 40 437-441 (1985)
7. D.G. PIPELEERS, P.A. IN`TVELD, M. VAN DE WINKEL, E. MAES, F.C. SCHUIT, and W. GEPTS, Endocrinology 117 806-817 (1985)
8. D.A. WILLIAMS, K.V. FOGARTY, R.Y. TSIEN, and F.S. FAY, Nature (Lond) 318

558-561 (1985)
9. B. DESBUQUOIS, and G.D. AURBACH, J. Clin. Endocrinol. Metab. 33 732-738 (1971)
10. D.B. DUNCAN, Biometrics 11 1-42 (1955)
11. K. KAWAI, and R.H. UNGER, Endocrinology 113 111-113 (1983)
12. J.E. GERICH, M.A. CHARLES, and G.M. GRODSKY, J. Clin. Invest. 54 833-841 (1974)
13. H. TANIGUCHI, K. MURAKAMI, M.YOSHIOKA, K. EJIRI, K. ISHIHARA, S. BABA, and Y. OKADA, Problems in GABA Research from Brain to Bacteria Okada Y, Roberts E (eds), 387-405 Excerpta Med., Amsterdam (1982)
14. J.C. GERBER, and T.A. HARE, Brain Res. Bull. 5 341-346 (1980)
15. A.REETZ, M. SOLIMENA, M. MATTEOLI, F. FOLLI, K. TAKEI, and P. DE CAMILLI, EMBO J. 10 1275-1284 (1991)
16. P.A. BAUMANN, P. WICKI, C. STIERLIN, and P.C. WALDMEIER, Naunyn-Schmiedeberg's Arch. Pharmacol. 341 88-93 (1990)
17. R.R. MATSUMOTO, Brain Res. Rev. 14 203-225 (1989)
18. S.CHARLES, T.TAMAGAWA, and J-C. HENQUIN, Biochem. J. 208 301-308 (1982)