# Inhibition of Insulin Secretion by Aryl-substituted Secondary Aminoethanols

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SUMMARY

**The administration of glucose, tolbutamide, ACTH, and glucagon to mice resulted in increase of plasma insulin levels. These increases were all inhibited by the prior administration of aryl-substituted secondary aminoethanols (ASAE). Cyclic 3′,5′ AMP resulted in increased levels of plasma insulin in intact mice, and this stimulatory effect was also inhibited by the aryl-substituted secondary aminoethanols. The locus of the inhibitory effect of these compounds on insulin secretion is discussed.** DIABETES *18*:262-67, May, 1969.

## INTRODUCTION

The cellular level of cyclic 3′,5′ adenosine monophosphate (cyclic 3′,5′ AMP) has been found to be an important regulating factor in a number of physiological processes.[1,2] The steady state level of the cyclic nucleotide reflects the activities of adenylcyclase,[3] which catalyses the synthesis of cyclic 3′,5′ AMP from ATP and $Mg^{++}$, and phosphodiesterase,[4] which catalyses the hydrolysis of cyclic 3′,5′ AMP to 5′ AMP.

Recent investigations have shown that the effect of a number of agents on insulin secretion may be mediated via α and β-adrenergic pancreatic islet cell receptors.[5,7] It has been found that β-adrenergic receptor stimulation increases, whereas α-adrenergic receptor stimulation decreases islet cell levels of cyclic 3′,5′ AMP.[6] The increased levels of cyclic nucleotide are thought to result in activation of a system involved in insulin secretion.[6,7] Sussman and Vaughan[8] have demonstrated the stimulatory effect of cyclic 3′,5′ AMP on insulin secretion in the isolated perfused rat pancreas, and Levine[9] has shown this stimulation to occur in man.

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In this communication, data will be presented to show that plasma levels of insulin in mice are increased by glucose and a number of pharmacological agents (tolbutamide, ACTH, glucagon and $N^6$-2′-O-Dibutyryl-cyclic-3′,5′ AMP (dibutyryl cyclic 3′,5′ AMP), and that the stimulatory effect of all these agents is inhibited by prior treatment of the animals with aryl-substituted secondary aminoethanols (ASAE), a class of compounds that includes β-adrenergic receptor blocking agents.[10]

## MATERIALS AND METHODS

White male mice weighing 20 to 25 gm. were offered a standard diet until twenty-four hours before use. Blood glucose was determined by the glucose oxidase method of Saifer and Gerstenfeld.[11] Plasma insulin assays were done by the procedure of Genuth, Frohman and Lebovitz.[12]

The following agents were generously supplied: (—), (+) and (±)-4-(2-isopropylamine-1-hydroxyethyl) methanesulfonanilide • HCl (MJ1999, Mead Johnson), (—), (+) and (±)-1-(isopropylamino)-3-(1-naphthyloxy)-2-propanol • HCl (propranolol, Inderal, Ayerst), Tolbutamide, Orinase, Upjohn, porcine corticotropin (ACTH, Upjohn), Glucagon (Pork-Beef mixture, Lilly). Dibutyryl cyclic 3′,5′ AMP was purchased from Calbiochem.

## RESULTS

*The effect of aryl-substituted secondary aminoethanols (ASAE) on the hypoglycemic response to tolbutamide.* The intraperitoneal (IP) administration of 5 mg. of tolbutamide resulted in a fall in blood glucose that was significant at fifteen minutes and maximal at forty-five to sixty minutes. The prior administration of either (±)-propranolol or (±)-MJ1999, which are both β-adrenergic receptor blocking agents,[13] obviated the hypoglycemic response to tolbutamide (figure 1).

*The effect of aryl-substituted secondary aminoethanols on plasma insulin responses to IP tolbutamide.* The IP administration of 5 mg. of tolbutamide to mice re-

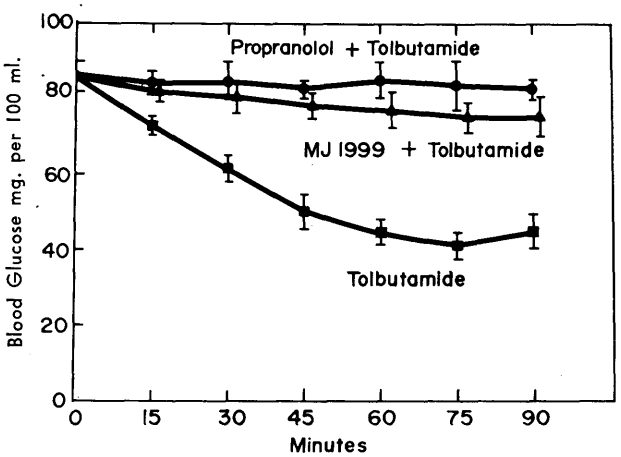


FIG. 1. The effect of MJ1999 and propranolol on the hypoglycemic response to tolbutamide. One group of mice received 0.4 mg. (±)-MJ1999 intraperitoneally sixty minutes prior to the injection of tolbutamide; a second group received 0.25 mg. (±)-propranolol, whereas the third group received saline. All groups were given 5 mg. of tolbutamide intraperitoneally and blood glucose was determined at the indicated times. Each time period represents the Mean ± SE of eight animals per group.

results in increased plasma insulin levels that are maximal at fifteen minutes (ten to twenty minute range). The prior administration of varying doses of either (±)-MJ1999 or (±)-propranolol resulted in a dose-related inhibition of tolbutamide-stimulated increases of plasma insulin. These data are shown in table 1. Significant inhibition of tolbutamide-stimulated increases of plasma insulin was obtained with both ASAE compounds at a dose of about 10 mg./kg. Doses of MJ1999 and propanolol less than 5 mg./kg. did not significantly reduce tolbutamide-stimulated increases of plasma insulin. A number of studies on β-adrenergic receptor blocking agents has been carried out in animals and man using a design of assessing the capacity of these agents to inhibit the effects of isoproterenol, a β-adrenergic stimulator, on parameters of contractile force of the heart, heart rate, blood pressure, uterine relaxation, and the like. These studies have shown that β-adrenergic receptor blockade is achieved by doses of (±)-propranolol and (±)-MJ1999 in the range of 0.1 to 0.5 mg./kg.[5,10,13-16]

*The effects of MJ1999 and propranolol on the plasma insulin response to intravenous tolbutamide.* The intravenous administration of 1 mg. of tolbutamide results in a rapid increase of plasma insulin in the mouse, which is maximal at 1.5 to three minutes, and returns to baseline levels at eight to twelve minutes. The data of figure 2 show that IP (±)-MJ1999 inhibited the

TABLE 1

The effects of aryl-substituted secondary aminoethanols (ASAE) on plasma insulin response to IP tolbutamide

| Dose of ASAE mg./kg. | (±)-Propranolol | Plasma Insulin* μU./ml. | (±)-MJ1999 |
|---|---|---|---|
| None | | 63±7 | |
| 1.0 | 66±3 | | 61±4 |
| 2.5 | 58±4 | | 55±8 |
| 5.0 | 49±8 | | 56±5 |
| 10.0 | 28±5 | | 40±4 |
| 20.0 | 20±6 | | 28±7 |
| 40.0 | 14±6 | | 19±3 |

*Mean ± SE of observation on six animals.

*The effect of MJ1999 and propranolol on tolbutamide stimulation of plasma insulin levels.* Animals were divided into three groups. At zero time, two of the groups received the indicated doses of either (±)-MJ1999 or (±)-propranolol IP, whereas the other group received saline. Sixty minutes later all three groups were given 5 mg. of tolbutamide IP. Plasma insulin was determined at fifteen minutes after tolbutamide administration.

IV tolbutamide-stimulated increases in plasma insulin at 1.5 and three minutes. This same magnitude of inhibition was obtained when 0.25 mg. IP (±)-propranolol was used instead of MJ1999 (data not shown). Figure 2 shows that there was a slight fall in blood glucose in response to tolbutamide between 1.5 and three minutes, but this was abolished by the prior administration of MJ1999.

*The effect of MJ1999 on the plasma insulin response to intravenous glucose.* The intravenous administration of 25 mg. of glucose into mice results in a rapid increase in plasma insulin that is maximal at 1.5 to three minutes. The data of figure 3 show that intravenous glucose resulted in a rise in plasma insulin that was still substantially elevated at three minutes. The prior administration (±)-MJ1999 resulted in a significant inhibition of the glucose-stimulated plasma insulin increase at 1.5 and three minutes. A similar magnitude of inhibition was obtained when 0.25 mg. of (±)-propranolol was used instead of MJ1999 (data not shown).

*The effect of MJ1999 on the plasma insulin response to intravenous corticotropin and glucagon.* Cyclic 3',5' AMP has been implicated as an intermediate in the mechanism of action in a number of peptide hormones, including corticotropin and glucagon.[1,2] Corticotropin and glucagon have also been found to stimulate rises in plasma insulin.[17,18] Because β-adrenergic stimulating agents augment plasma insulin levels and because corticotropin and glucagon also do this, as well as cause the activation of adenylcylase in the adrenals, liver, and adipose tissue, β-adrenergic receptor blockade of pep-

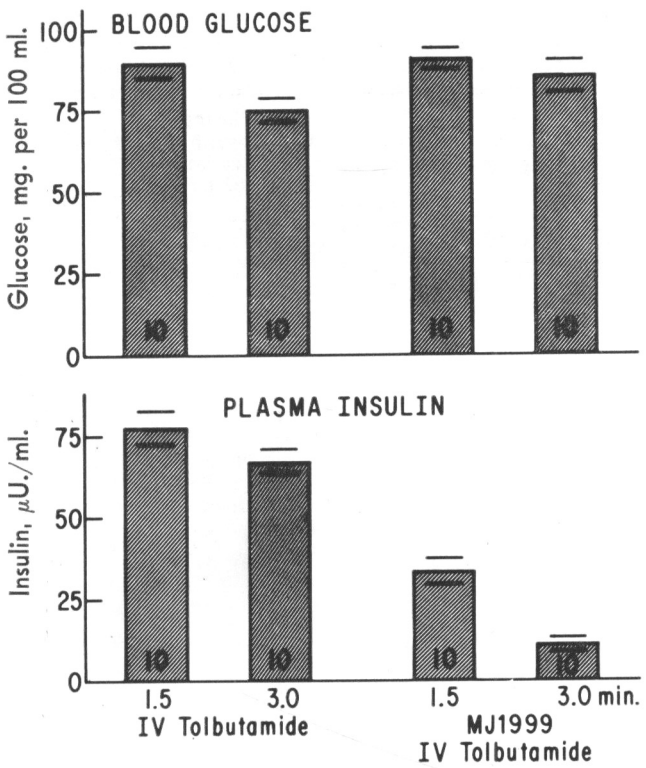


FIG. 2. The effect of MJ1999 on the plasma response to intravenous tolbutamide. Mice were divided into two groups, one of which received 0.50 mg. (±)-MJ1999 intraperitoneally sixty minutes prior to the start of the experiment, whereas the other group received saline. At zero time, all animals were given one mg. of tolbutamide intravenously. Blood glucose and plasma insulin were determined at 1.5 and three minutes. Each group represents the Mean ± SE of observations on ten animals.

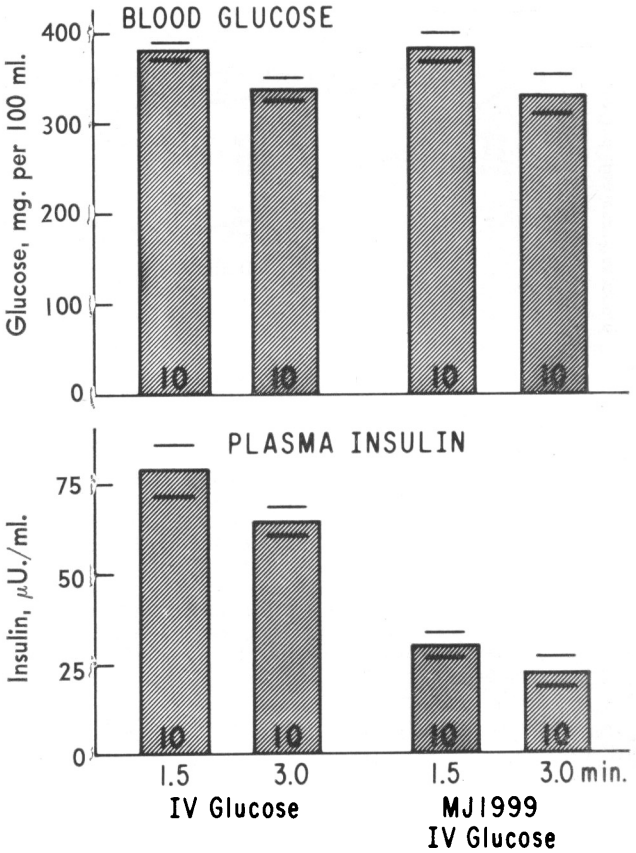


FIG. 3. The effect of MJ1999 on the insulin response to intravenous glucose. Protocol as described in figure 2, except for the use of 25 mg. of intravenous glucose instead of tolbutamide. Each group represents the Mean ± SE of observations on ten animals.

tide-stimulated insulin secretion was investigated. The data of figure 4 show that both IV ACTH and glucagon increased plasma insulin levels and that the prior administration of (±)-MJ1999 inhibited the stimulation by both of these hormones.

*The effect of dextro and levo propranolol on tolbutamide-stimulated insulin secretion.* Studies on β-adrenergic receptor blocking agents have shown that the blocking effect is exerted by the levo isomer, whereas the dextro form has little activity.[19,20] The data of table 2 show that (+) and (—)-propranolol were equally potent inhibitors of IV tolbutamide-stimulated insulin release at 1.5 and three minutes. Both isomers exerted marked inhibitory effects. Similar magnitudes of inhibition of IV tolbutamide-stimulated increases in plasma insulin levels were obtained with (+) and (—)-MJ1999 (data not shown).

*The effect of MJ1999 on the plasma insulin response to intravenous cyclic 3′,5′ AMP.* The intravenous administration of dibutyryl cyclic 3′,5′ AMP to mice resulted in a marked increase of plasma insulin levels that were maximal at ten to twelve minutes. These levels were further augmented by the addition of intravenous aminophyllin. The prior administration (±) of MJ1999 to the animals inhibited both the aminophyllin and the cyclic 3′,5′ AMP-stimulation of plasma insulin levels. These data are shown in figure 5.

## DISCUSSION

β-adrenergic receptor stimulating agents have been shown to cause increased insulin secretion in vitro and in vivo,[5,6] and to augment levels of cyclic 3′,5′ AMP in the pancreatic islet β-cells.[6] These effects are abolished by pretreatment with a β-adrenergic receptor blocking agent.[5,6] It has been found that cyclic 3′,5′ AMP itself stimulates insulin secretion.[8,9] Two mechanisms have been suggested to account for the stimulatory effect of

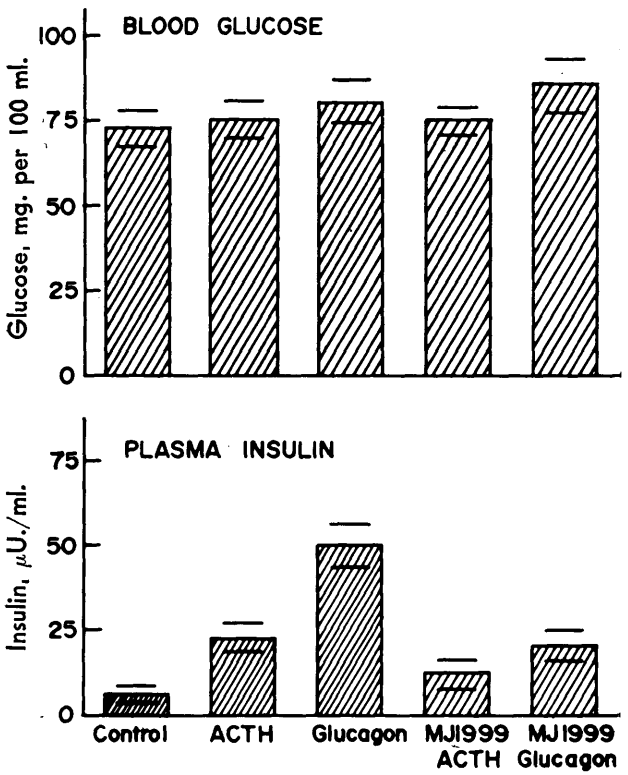


FIG. 4. The effect of MJ1999 on the plasma insulin response to intravenous corticotropin and glucagon. Mice were divided into five groups. At minus sixty minutes, two groups received 0.6 mg. (±)-MJ1999 intraperitoneally, whereas the other three received saline. At zero time, two groups of animals (one MJ1999, one saline) were given 10 μg of intravenous corticotropin, two other groups (one MJ1999, one saline) received 25 μg of intravenous glucagon, and one group (saline) received an equal volume (0.1 ml.) of intravenous saline. Blood glucose and plasma insulin were determined at ten minutes. Each group represents the Mean ± SE of observations on six animals.

the cyclic nucleotide on insulin secretion. Malaisse and co-workers have postulated that the pancreatic β-cell contains an adenylcylase system through which phosphorylase is activated and have suggested that insulin secretion might depend on and be controlled by hormones and other pharmacological agents that influence the glycolytic rate in the β-cell.[7] Rasmussen and Tennenhouse[21] have attributed the stimulatory effect of cyclic 3',5' AMP to its alteration of the permeability of cell membranes to calcium. The absolute requirement for extracellular calcium in insulin secretion has been demonstrated in the isolated perfused rat pancreas and in pieces of rabbit pancreas.[22,23]

The data presented in this communication show that plasma levels of insulin are increased by agents (ACTH, glucagon) that are known to elevate levels of cyclic

TABLE 2

The effect of dextro and levo propranolol on tolbutamide-stimulated insulin secretion

| | Blood glucose* | Plasma insulin |
|---|---|---|
| | mg.% | μU./ml. |
| Controls | 83±5 | 7±2 |
| Tolbutamide 1.5 minutes | 80±8 | 68±8 |
| (+)-Propranolol then tolbutamide 1.5 minutes | 88±5 | 23±8 |
| (—)-Propranolol then tolbutamide 1.5 minutes | 77±8 | 28±7 |
| Tolbutamide 3 minutes | 71±6 | 58±6 |
| (+)-Propranolol then tolbutamide 3 minutes | 90±5 | 18±5 |
| (—)-Propranolol then tolbutamide 3 minutes | 83±7 | 20±8 |

*Mean ± SE of observation on eight animals.

*The effect of* (+) *and* (—)*-propranolol on tolbutamide-stimulated insulin secretion.* Protocol as described in figure 2, except for the use of 0.4 mg. of (+) or (—)-propranolol instead of 0.5 mg. (±)-MJ1999.

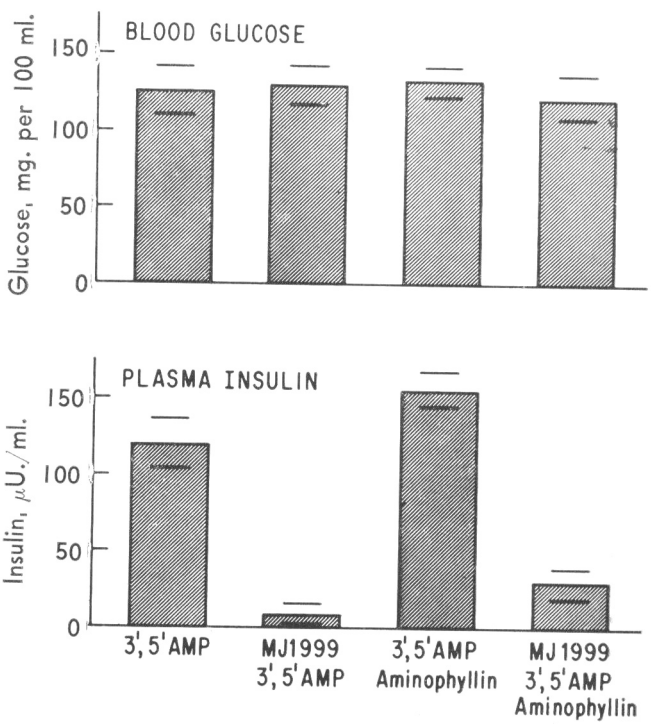


FIG. 5. The effect of MJ1999 on the plasma insulin response to intravenous cyclic 3',5' AMP. Mice were divided into four groups. Two groups received 0.5 mg. (±)-MJ1999 intraperitoneally at minus sixty minutes, whereas the other two groups received saline. At zero time, one MJ1999 and one saline group received one mg. of cyclic 3',5' AMP intravenously, whereas the other two groups (one MJ1999, one saline) received 1 mg. cyclic 3',5' AMP plus 1 mg. aminophyllin intravenously. Blood glucose and plasma insulin were determined at ten minutes. Each group represents the Mean ± SE of observations on eight animals.

3',5' AMP in tissues other than the pancreatic islets,[2] and by glucose and tolbutamide, the effects of which on tissue levels of cyclic 3',5' AMP have not yet been reported. The stimulatory effects of these agents on plasma insulin levels were inhibited by large doses of racemic propranolol and MJ1999. The inhibitory effect of these aryl-substituted secondary aminoethanols was obtained at doses which are twenty to two-hundred times that needed to achieve β-adrenergic receptor blockade in animals and man. Studies on β-adrenergic receptor blocking agents have shown that the adenylcyclase blocking effect is exerted by the levo form, whereas the dextro form has little activity.[19,20] However, the dextro and levo forms of both propranolol and MJ1999 were equipotent in inhibiting tolbutamide-stimulated increases of plasma insulin.

Animal experiments have suggested that blockade of β-adrenergic receptors does not explain the capacity of β-adrenergic receptor blocking agents to abolish cardiac arrhythmias.[10,20,24-26] It has been found that (+) and (±)-propranolol are equipotent as anti-arrhythmia agents and that MJ1999 has cardiodepressant activity in reserpinized cats.[20,24-26] The local anesthetic property of propranolol and other ASAE compounds has been demonstrated and the levo and dextro forms found to be equally active.[10,20] It has been suggested that the local anesthetic or "quinidine-like" activity of the aryl-substituted secondary aminoethanols may play an important role in the anti-arrhythmic properties of these compounds.[10,20,24-26] This local anesthetic or "quinidine-like" activity of the ASAE compounds does not correlate well with their potency as β-adrenergic receptor blocking agents.[10,24,26] The "quinidine-like" activity is, however, obtained at higher dose levels than those needed to achieve β-adrenergic receptor blockade.[10,14]

Jaanus, Miele and Rubin have demonstrated the inhibitory effects of propranolol and other local anesthetics on the calcium-stimulated release of catecholamines from the perfused cat adrenal medulla.[27] The inhibitory effect of propranolol was partially overcome by increasing the calcium concentration in the perfusing solution. These investigators have suggested that the inhibition of catecholamine secretion caused by propranolol is due to its local anesthetic activity, which interferes with calcium movement across cell membranes.

The failure of dibutyryl cyclic 3',5' AMP to overcome the inhibition of insulin secretion resulting from ASAE compounds places at least part of the inhibition beyond adenylcyclase. The inhibition of glucose and drug-stimulated insulin secretion by these compounds may not be due to decreased formation of the cyclic nucleotide alone, but may also involve an inhibition of the stimulatory effect of cyclic 3',5' AMP on the mechanism of insulin secretion. Moreover, the equipotent inhibitory effects of the levo and dextro isomers of propranolol and MJ1999 suggest that the latter mechanism may be the more important.

Recent reports suggest that β-adrenergic receptor blocking agents influence processes that maintain the blood glucose in animals and man. The administration of propranolol was found to decrease the hypoglycemic response to intravenous tolbutamide in man.[28] This could be explained by the data presented in this communication (cf. figure 2); however, the propranolol dose used in the experiments on mice was much greater (man, 0.15 mg./kg., mice, 10.0 mg./kg.).

It has been found that β-adrenergic blocking agents may result in hypoglycemia in diabetic patients on insulin therapy[29,30] and in normal man.[31] Propranolol has also been shown to reduce the rise in blood glucose that follows exercise in man.[32] It has been suggested that β-adrenergic receptor blocking agents interfere with glycogenolytic, lipolytic, and gluconeogenic actions of catecholamines, which serve to maintain the blood glucose.[33] These responses might be caused by an inhibition of adenylcyclase stimulation and some interference with the end organ effects of cyclic 3',5' AMP.

## ACKNOWLEDGMENT

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