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# SHORT COMMUNICATIONS

## M&B 39890A, a novel sulphonamido-benzamide compound, inhibits insulin and glucagon secretion *in vitro*

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It was recently reported that the compound *N*-(3-imidazol-1-ylpropyl)-2-(3-trifluoromethylbenzenesulphonamido) benzamide hydrochloride (M&B 39890A), one of a series of novel sulphonamido-benzamides, reduces fasting glycaemia in genetically obese mice (C57BL6J ob/ob strain) without affecting circulating insulin levels [1]. Furthermore, in isolated rat islets of Langerhans, M&B 39890A was found to inhibit arginine-induced glucagon secretion as well as to potentiate the suppressor effect of glucose on glucagon release, this without modifying insulin output [1].

To gain further insight into the influence of M&B 39890A, on islet cell function, we have investigated the effect of this substance on the release of insulin, glucagon and somatostatin by the isolated perfused rat pancreas. M&B 39890A was tested in conditions of unstimulated hormone release as well as in the presence of arginine and vasoactive intestinal peptide (VIP), both of which are effective secretagogues of pancreatic B, A and D cells [2–4].

*Materials and methods*

Fed male Wistar rats (200–225 g body weight) were used as donors. The pancreas was dissected and perfused *in situ* according to the procedure of Leclercq-Meyer *et al.* [5] as adapted in our laboratory [3]. Effluent samples were collected from the portal vein, without recycling, at 2-min intervals (flow rate, 2 ml/min) in tubes containing 2000 KIU Trasylol (Bayer AG, Leverkusen, F.R.G.), and frozen at −20° until the time of assay.

The perfusion medium consisted of a Krebs–Henseleit buffer (gas phase 95:5, $O_2$:$CO_2$; pH 7.4) supplemented with 4% (w/v) dextran T-70, 0.5% (w/v) bovine albumin (Cohn fraction V) and glucose (5.5 mmol/l). After a 35-min equilibration period, baseline samples were collected for 12 min. At zero time, M&B 39890A was infused through a sidearm cannula as a priming dose (400 ng in 1 min), followed by constant infusion at a rate of 10.5 μg/min (30 μM) for 16 or 20 min. As secretagogues of the endocrine pancreas, L-arginine hydrochloride (Sigma Chemical Co.,

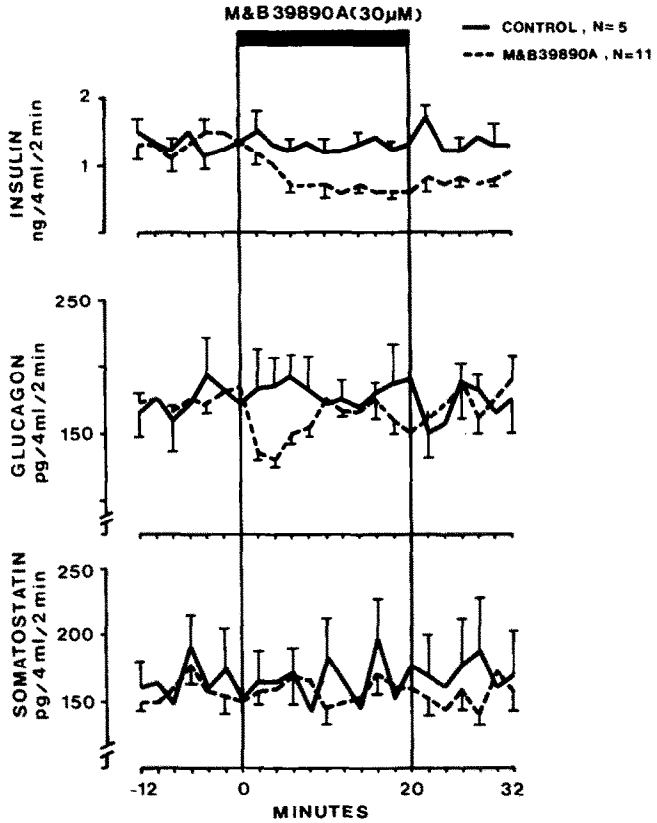


Fig. 1. Effect of M&B 39890A (30 μM) on unstimulated insulin, glucagon and somatostatin release by the perfused rat pancreas (means ± SEM). Solid and broken lines correspond to control and M&B 39890A experiments, respectively.

St Louis, MO) and VIP (Peninsula Laboratories, Belmont, CA) were employed. Addition of these substances to the perfusate was performed as described in the figures.

Radioimmunoassay was employed to measure insulin [6], glucagon [7] and somatostatin [8]. Antiglucagon serum (30K) and antisomatostatin serum (80C) were kindly donated by Dr R. H. Unger (University of Texas Health Sciences Center at Dallas, Texas). All samples for each series of experiments were analyzed in the same run.

Results are presented as the mean ± SEM. Hormone response, from zero time until the end of secretagogue infusion, was calculated as the integrated area of the curve above or below the mean preinfusion level (average of all baseline levels), using the trapezoidal method. Differences between values were tested for significance by analysis of variance and by the Student's *t*-test for unpaired samples.

*Results and discussion*

As shown in Fig. 1, addition of M&B 39890A to the perfusate induced a depression of unstimulated insulin secretion which persisted upon removal of the drug ($F_{10,100} = 6.23$; $P < 0.01$). Furthermore, during M&B 39890A infusion, insulin output, as calculated by the integrated area under the response curve, was reduced as compared to control perfusions (15.9 ± 2.4 ng/20 min vs 25.6 ± 3 ng/20 min; $P = 0.033$). M&B 39890A evoked a transient reduction of glucagon release ($F_{10,100} = 4.8$; $P < 0.01$). The integrated area under the response curve (3,156 ± 107 pg/20 min) was significantly smaller than that corresponding to the control experiments (3,580 ± 126 pg/20 min; $P = 0.035$). M&B 39890A did not significantly affect somatostatin secretion.

Figure 2 demonstrates that the release of insulin elicited by arginine (incremental response, 238 ± 39 ng/20 min) was markedly inhibited by M&B 39890A (incremental response: 117 ± 17 ng/20 min; $P = 0.031$). M&B 39890A also blocked the glucagon response to arginine (incremental response: 6,734 ± 2,122 pg/20 min vs 18,927 ± 2,924 pg/20 min in control experiments; $P = 0.011$); it did not significantly modify somatostatin output induced by this amino acid.

In accordance with the previous series of experiments, and as reflected in Fig. 3, M&B 39890A markedly blocked the insulin secretion evoked by VIP (incremental response: 81 ± 13 ng/16 min vs 163 ± 20 ng/16 min in control perfusions; $P = 0.005$) as well as the release of glucagon induced by this peptide (incremental response:

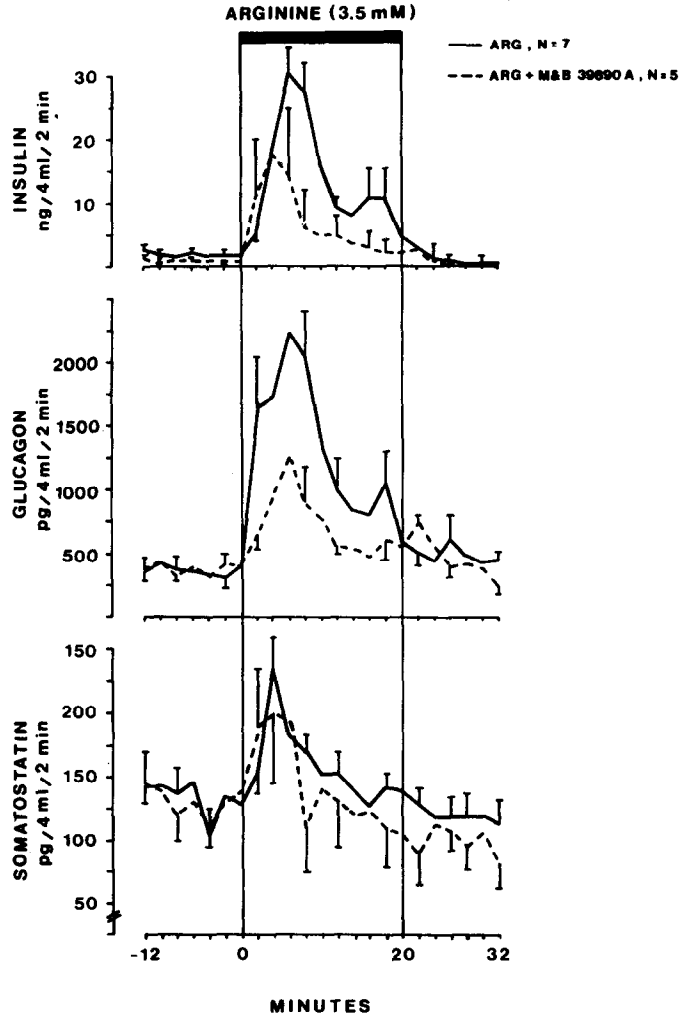


Fig. 2. Effect of M&B 39890A (30 μM) on the insulin, glucagon and somatostatin responses to arginine (3.5 mM) by the perfused rat pancreas (means ± SEM). Solid line represents arginine experiments. Broken line represents arginine plus M&B 39890A experiments.

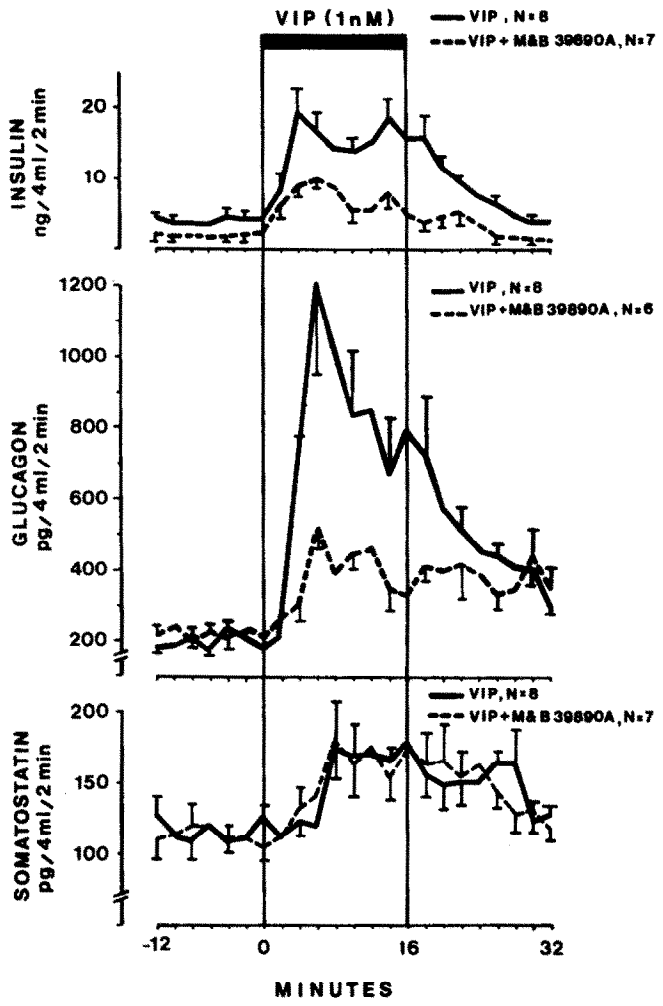


Fig. 3. Effect of M&B 39890A (30 $\mu$M) on the insulin, glucagon and somatostatin responses to VIP (1 nM) by the perfused rat pancreas (means ± SEM). Solid line represents VIP experiments. Broken line represents VIP plus M&B 39890A experiments.

2,700 ± 314 pg/16 min vs 8,752 ± 2,194 pg/16 min in control perfusions; P = 0.037). Again, M&B 39890A had no effect on somatostatin output.

In agreement with the report of Tadayyon *et al.* [1], our present data show that, in the perfused rat pancreas, M&B 39890A inhibits unstimulated glucagon release as well as the secretory response of this hormone to arginine. Furthermore, our results demonstrate that M&B 39890A suppresses glucagon output as elicited by VIP.

At the concentration employed in our experiments (30 $\mu$M), M&B 39890A behaved as an effective inhibitor of insulin release, both in the absence and in the presence of B-cell secretagogues (arginine and VIP). In fact, the blocking effect of M&B 39890A on unstimulated insulin secretion persisted upon discontinuance of the infusion of this drug. Although Tadayyon *et al.* [1] found no effect of M&B 39890A on plasma insulin levels in genetically obese mice, these investigators did observe that this compound slightly reduced arginine-induced insulin secretion in isolated rat Langerhans islets.

In our rat pancreas system, M&B 39890A did not significantly modify somatostatin output under any of the experimental conditions examined.

The effects of M&B 39890A on islet cell function resemble those of diazoxide, another benzo-sulphonamide, which also blocks insulin and glucagon secretion [9, 10]. This supports the suggestion that a common radical of both compounds is responsible for the inhibition of B- and A-cell secretion. The sulfonylureas, chemical congeners of these substances, also share with them the ability to block glucagon release [3, 11, 12] while exerting an opposite effect on insulin output.

Intra-islet glucagon has been postulated to stimulate, in a paracrine fashion, the release of insulin [13]. However, the depression of insulin secretion caused by M&B 39890A does not seem to be a consequence of reduced glucagon output since in unstimulated conditions, i.e. in the absence of secretagogues, the inhibition of insulin and glucagon secretion induced by this drug followed a different pattern, the latter being manifest only during the initial period of M&B infusion.

In summary, M&B 39890A, at 30 $\mu$M, inhibited unstimulated insulin and glucagon output; it also markedly blocked the secretory responses of these hormones to 3.5 mM arginine and to 1 nM VIP, but did not significantly affect somatostatin secretion under any of the experimental conditions examined. M&B 39890A represents a new experimental tool to manipulate the secretion of the endocrine

pancreas. Its inhibition of glucagon release encourages the search for related compounds devoid of effect on the B-cell as a therapeutical means to reduce the hyperglucagonism associated with diabetes mellitus [14].

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*Hospital Puerta de Hierro*
*Universidad Autónoma de Madrid*
*Madrid*
*Spain*

RAMONA A. SILVESTRE
ELENA PEIRÓ
PALOMA MIRALLES
JOSÉ MARCO

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Correspondence to: Dr J. Marco, Hospital Puerta de Hierro, Universidad Autónoma de Madrid, San Martín de Porres, 4, 28035 Madrid, Spain.

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# Reduction of doxorubicin toxicity by methylene blue in cultured rat myocardial cells

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The antitumor anthracycline doxorubicin (DOX) is a widely used antineoplastic agent effective in the treatment of a variety of human cancers [1]. However, its clinical use is associated with dose-limiting acute and chronic cardiotoxic effects [2]. The mechanism of DOX cardiotoxicity, though not completely understood, is thought to involve the generation of reactive oxygen species resulting from NAD(P)H-dependent reduction of DOX to its semiquinone free radical form [3, 4]. Doroshow [5] has shown that rat cardiac sarcosomal, mitochondrial, and cytosolic fractions generate superoxide in the presence of a variety of anthracyclines in an NAD(P)H-dependent manner even in the presence of oxygen radical detoxifying enzymes. He proposed that the generation of reactive oxygen species in excess of the detoxification capabilities of the myocardium is the mechanism of importance in anthracycline cardiotoxicity.

Methylene blue (MB) is a redox dye which is capable of oxidizing NAD(P)H in biological systems [6, 7]. Hrushesky *et al.* [7] postulated that MB administered concurrently with DOX would affect the concentration of intracellular reducing agents, prevent the reduction of DOX, and thus protect the myocardium from DOX-mediated damage. MB reduced cardiotoxicity in the mouse yet the antitumor activity of DOX was not affected in the tumor model studied [7].

In the present study, beating rat myocardial cells in culture were used to examine further the protective effects