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FACILITATION OF INSULIN RELEASE BY *N*-*p*-TOSYLGLYCINE

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Abstract—*N*-*p*-tosylglycine, which inhibits transglutaminase activity in islet homogenates, was found to cause a rapid and sustained facilitation of insulin release evoked by D-glucose, L-leucine or the association of Ba^{2+} and theophylline in intact islets. Such a facilitating action could not be attributed to any obvious effect upon either nutrient oxidation or ^{45}Ca net uptake and outflow. It failed to be reproduced by glycine, *Nα*-*p*-tosyl-L-arginine methyl ester or *Nα*-*p*-tosyl-L-lysine methyl ester. *N*-*p*-tosylglycine (5.0 mM) slightly enhanced insulin release evoked by a high concentration of glucose (16.7 mM) and failed to affect significantly the secretory response to the association of L-leucine and L-glutamine or that of D-glucose and gliclazide. *N*-*p*-tosylglycine failed to affect the incorporation of [2,5-^{3}H]histamine in trichloroacetic acid-precipitable material in intact islets. These results suggest that *N*-*p*-tosylglycine interferes with a late event in the secretory sequence, possibly at the level of the cell boundary, rather than inhibiting the crosslinking of intracellular proteins.

Pancreatic islets display Ca^{2+}-responsive transglutaminase activity [1, 2]. The possible participation of this enzyme, which catalyzes the crosslinking of proteins, in the process of insulin release remains to be unambiguously documented. Indeed, prior studies dealing with several inhibitors of the enzyme have failed to provide conclusive information. For instance, extensive studies on the metabolic, ionic, biosynthetic and secretory effects of methylamine, dimethylamine and trimethylamine in intact pancreatic islets have indicated that the inhibitory effects of these agents upon insulin release is not necessarily tightly related to inhibition of transglutaminase, as documented in islet homogenates [3–5]. Likewise, considerable difficulties were encountered in the interpretation of experimental data obtained when other inhibitors of transglutaminase activity, namely monodansylcadaverine, bacitracin and hypoglycaemic sulphonylureas, were examined for their effects upon the functional behaviour of intact islets [1, 6, 7]. Pursuing our efforts to assess the possible role of transglutaminase in islet function, we have investigated, in the present study, the effect of *N*-*p*-tosylglycine upon insulin release by isolated pancreatic islets. This agent was already shown to inhibit transglutaminase activity in islet homogenates [1] like in other tissues [8]. The present results demonstrate that, unexpectedly, *N*-*p*-tosylglycine facilitates insulin release evoked by several secretagogues.

MATERIALS AND METHODS

All experiments were performed with pancreatic islets isolated [9] from the pancreas of fed albino rats. The methods used to measure transglutaminase activity in islet homogenates [1]; insulin release [10], ^{45}Ca net uptake [11], nutrient oxidation [12] and [2,5-^{3}H]-histamine incorporation in TCA-precipitable material [4] in incubated islets; and ^{45}Ca outflow [13] and insulin release [14] from perifused islets were all described in prior publications. For reasons given elsewhere [10], the statistical comparison between mean insulin secretory rates is always restricted to data collected within the same experiment(s). Hence, the control values (e.g. those collected in the sole presence of D-glucose) may vary from one set of experiments to another. In Table 2, however, all relevant measurements derived from the present experiments were pooled together to provide an overall estimation of the secretory response to *N*-*p*-tosylglycine.

N-*p*-tosylglycine, *Nα*-*p*-tosyl-L-arginine methyl ester and *Nα*-*p*-tosyl-L-lysine methyl ester were purchased from Sigma Chemicals Company (St. Louis, MO, U.S.A.).

All results are expressed as the mean (±S.E.M.) together with the number of individual observations (N), the statistical significance of differences between mean values being assessed by use of Student's *t*-test.

RESULTS

Enzymic data

At a fixed concentration of 5.0 mM, *N*-*p*-tosylglycine was a more powerful inhibitor of transglutaminase activity in islet homogenates than either *Nα*-*p*-tosyl-L-lysine methyl ester or *Nα*-*p*-tosyl-L-arginine methyl ester (Table 1). We have previously characterized the dose–action relationship for the inhibitory action of *N*-*p*-tosylglycine upon transglutaminase activity in islet homogenates [1].

In intact islets incubated for 60 min in the absence of exogenous nutrient, *N*-*p*-tosylglycine (5.0 mM) failed to affect significantly the incorporation of [2,5-^{3}H]histamine (0.2 mM) in trichloroacetic acid-pre-

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Table 1. Effect of *N-p*-tosylglycine and other agents upon transglutaminase activity

Agent (mM)	Reaction velocity (% of control)
N-p-tosylglycine (5.0)	12.6 ± 2.0 (6)
Nα-p-tosyl-L-lysine methyl ester (5.0)	56.9 ± 5.0 (6)
Nα-p-tosyl-L-arginine methyl ester (5.0)	82.3 ± 2.6 (6)

All measurements were performed in the presence of [2,5-^{3}H]-histamine and Ca^{2+} (0.5 mM each), the control value averaging 10.1 pmoles/30 min per islet.

cipitable material. Such an incorporation averaged 17.8 ± 2.6 and 16.1 ± 0.5 fmoles/60 min per islet (N = 5 in each case) in the absence and presence of *N-p*-tosylglycine, respectively.

Secretory data

N-p-tosylglycine (5.0 mM) failed to affect significantly basal insulin output but augmented insulin release evoked by D-glucose (Table 2). In absolute terms, the increment in insulin output evoked by *N-p*-tosylglycine (5.0 mM) was most marked at an intermediate glucose concentration (8.3 mM) but still present ($P < 0.05$) at a higher glucose level (16.7 mM). The 5.0 mM concentration of *N-p*-tosylglycine used in most experiments was not quite sufficient to provoke a maximal facilitation of insulin release, especially at an intermediate concentration of glucose (Table 2).

N-p-tosylglycine did not always enhance insulin release. Thus, whereas *N-p*-tosylglycine augmented ($P < 0.001$) leucine-induced like glucose-induced insulin secretion, it failed to affect ($P > 0.2$) insulin output in the presence of the combination of either L-leucine and L-glutamine or D-glucose and gliclazide (Table 3).

The data illustrated in Table 2 could suggest that *N-p*-tosylglycine acts as a nutrient in islet cells, e.g. through liberation of glycine. We have examined, therefore, the effect of *N-p*-tosylglycine upon the process of insulin release evoked, in the absence of Ca^{2+}, by the association of Ba^{2+} and theophylline (Table 4). Such a process is known to be exquisitely sensitive to nutrient secretagogues [15]. *N-p*-tosylglycine indeed augmented ($P < 0.001$) insulin output induced by a Ba^{2+} and theophylline. However, glycine itself failed to mimic the enhancing action of *N-p*-tosylglycine. Likewise, in the presence of glucose (7.0 mM), *N-p*-tosylglycine (10.0 mM) was much more potent than glycine (used at the same concentration) in augmenting insulin release (Table 5, Expt. 1).

Neither *Nα-p*-tosyl-L-arginine methyl ester nor *Nα-p*-tosyl-L-lysine methyl ester reproduced the stimulant action of *N-p*-tosylglycine upon glucose-stimulated insulin release (Table 5, Expt. 2).

The release of insulin evoked by the association

Table 2. Effect of *N-p*-tosylglycine upon insulin release evoked by D-glucose

N-p-tosylglycine	Nil	5.0 mM	10.0 mM
Nil	10.0 ± 1.5 (40)	13.1 ± 1.1 (22)	
D-glucose (5.6 mM)	14.6 ± 2.2 (19)	26.5 ± 2.3 (19)	
D-glucose (7.0 mM)	29.1 ± 1.7 (97)	54.8 ± 2.7 (95)	75.4 ± 5.4 (40)
D-glucose (8.3 mM)	52.3 ± 5.9 (17)	103.6 ± 7.5 (18)	
D-glucose (11.1 mM)	152.8 ± 16.2 (19)	189.5 ± 15.6 (19)	
D-glucose (16.7 mM)	193.9 ± 8.9 (40)	221.5 ± 9.3 (40)	236.2 ± 18.8 (20)

All results are expressed as μU/90 min per islet.

Table 3. Effect of *N-p*-tosylglycine upon insulin release evoked by amino acids and a sulphonylurea

Secretagogues (mM)	*N-p*-tosylglycine	
	Nil	5.0 mM
Nil	10.0 ± 1.5 (40)	13.1 ± 1.1 (22)
L-leucine (5.0)	32.8 ± 3.7 (20)	61.4 ± 4.5 (20)
L-leucine (10.0)	73.3 ± 7.4 (41)	102.1 ± 7.0 (41)
L-leucine (10.0) + L-glutamine (10.0)	163.3 ± 7.5 (21)	159.2 ± 8.7 (21)
D-glucose (7.0)	34.1 ± 3.1 (26)	80.6 ± 8.1 (27)
D-glucose (7.0) + gliclazide (0.06)	73.0 ± 6.0 (26)	83.3 ± 7.4 (28)

All results are expressed as μU/90 min per islet.

Table 4. Effect of *N*-*p*-tosylglycine and glycine upon insulin release evoked by Ba^{2+} and theophylline

Ca^{2+} (mM)	Ba^{2+} (mM)	Theophylline (mM)	*N*-*p*-tosylglycine (mM)	Glycine (mM)	Insulin output (μU/90 min per islet)
1.0	–	–	–	–	11.0 ± 3.2 (18)
–	2.0	1.4	–	–	49.5 ± 6.6 (19)
–	2.0	1.4	5.0	–	85.4 ± 7.1 (19)
–	2.0	1.4	–	5.0	53.4 ± 5.2 (19)

of D-glucose and *N*-*p*-tosylglycine was severely inhibited by menadione, antimycin A, in the absence of Ca^{2+} or presence of methylamine, and potentiated by either theophylline or cytochalasin B (Table 5, Expt. 3). This suggests that, like in the sole presence of glucose, the release of insulin facilitated by *N*-*p*-tosylglycine represented an energy- and Ca^{2+}-dependent process, involving both a change in redox state and activation of the microfilamentous effector system. Incidentally, even in the presence of D-glucose (7.0 mM) and either theophylline (1.4 mM) or cytochalasin B (0.02 mM), *N*-*p*-tosylglycine (5.0 mM) augmented insulin output. Thus, the increment in secretory rate attributable to *N*-*p*-tosylglycine, when expressed relative to the mean corresponding control value found in its absence, averaged 62.6 ± 9.9% (d.f. = 38; $P < 0.001$) in the presence of glucose and theophylline and 86.5 ± 10.7% (d.f. = 38; $P < 0.001$) in the presence of glucose and cytochalasin B. Methylamine inhibited ($P < 0.001$) insulin release evoked by the combination of D-glucose and *N*-*p*-tosylglycine, whether at an intermediate (7.0 mM) or high (16.7 mM) glucose concentration. However, in the presence of glucose and methylamine, *N*-*p*-tosylglycine still enhanced ($P < 0.001$) insulin output (Table 5, Expt. 4).

Cationic and metabolic data

N-*p*-tosylglycine (5.0 mM) failed to affect significantly ($P > 0.4$) basal or glucose-stimulated ^{45}Ca net uptake, and either D-(U-^{14}C]glucose or L-[U-^{14}C]leucine oxidation (Table 6).

In islets prelabelled with ^{45}Ca and perifused in the presence of D-glucose (7.0 mM) at normal Ca^{2+} concentration (1.0 mM), *N*-*p*-tosylglycine failed to exert any marked effect upon ^{45}Ca fractional outflow rate (Fig. 1). At the most, there was a trend towards a modest increase in ^{45}Ca outflow. In the same experiments, the administration of *N*-*p*-tosylglycine provoked an immediate and biphasic enhancement of insulin release. Over 30 min exposure to the drug (min 45–74 inclusive), the increment in insulin output averaged 0.13 ± 0.05 μU/min per islet ($N = 6$; $P < 0.05$), as computed by planimetry and after correction for the mean secretory rate observed prior to (min 42–44) and after (min 78–90) administration of *N*-*p*-tosylglycine. Such an increase was not observed in four control experiments performed throughout in the absence of *N*-*p*-tosylglycine (data not shown).

DISCUSSION

The present results reveal that *N*-*p*-tosylglycine

Table 5. Modulation of glucose-induced insulin release by *N*-*p*-tosylglycine and other agents

Expt.	Secretagogue(s) (mM)	Insulin output (μU/90 min per islet)
1.	D-glucose (7.0)	15.6 ± 1.8 (20)
	D-glucose (7.0) + glycine (5.0)	27.1 ± 3.6 (19)
	D-glucose (7.0) + glycine (10.0)	27.5 ± 3.9 (20)
	D-glucose (7.0) + *N*-*p*-tosylglycine (10.0)	90.6 ± 7.0 (20)
2.	D-glucose (7.0)	33.4 ± 4.7 (17)
	D-glucose (7.0) + *N*-*p*-tosylglycine (5.0)	80.9 ± 5.7 (18)
	D-glucose (7.0) + Nα-*p*-tosyl-L-arginine methyl ester (5.0)	36.4 ± 3.2 (18)
	D-glucose (7.0) + Nα-*p*-tosyl-L-lysine methyl ester (5.0)	31.6 ± 4.0 (18)
3.	D-glucose (7.0) + *N*-*p*-tosylglycine (5.0)	47.9 ± 3.0 (58)
	D-glucose (7.0) + *N*-*p*-tosylglycine (5.0) + menadione (0.025)	10.0 ± 1.5 (20)
	D-glucose (7.0) + *N*-*p*-tosylglycine (5.0) + antimycin A (0.01)	16.5 ± 1.6 (19)
	D-glucose (7.0) + *N*-*p*-tosylglycine (5.0) + EGTA (0.5), no $CaCl_2$	22.1 ± 3.4 (20)
	D-glucose (7.0) + *N*-*p*-tosylglycine (5.0) + methylamine (2.0)	15.1 ± 1.7 (20)
	D-glucose (7.0) + *N*-*p*-tosylglycine (5.0) + theophylline (1.4)	110.7 ± 12.1 (21)
	D-glucose (7.0) + *N*-*p*-tosylglycine (5.0) + cytochalasin B (0.02)	86.7 ± 7.4 (21)
4.	D-glucose (16.7)	141.5 ± 14.6 (20)
	D-glucose (16.7) + *N*-*p*-tosylglycine (5.0)	153.2 ± 13.1 (20)
	D-glucose (16.7) + methylamine (2.0)	33.5 ± 7.9 (20)
	D-glucose (16.7) + methylamine (2.0) + *N*-*p*-tosylglycine (5.0)	78.4 ± 8.7 (20)

Table 6. Effects of *N-p*-tosylglycine upon ^{45}Ca net uptake and nutrient oxidation in pancreatic islets

Nutrient (mM)	*N-p*-tosylglycine	
	Nil	5.0 mM
^{45}Ca net uptake (pmoles/90 min per islet)		
Nil	1.90 ± 0.20 (10)	1.75 ± 0.25 (10)
D-glucose (7.0)	2.66 ± 0.27 (10)	2.74 ± 0.29 (11)
Nutrient oxidation (pmoles/120 min per islet)		
D-[U-^{14}C]glucose (7.0)	13.0 ± 1.1 (9)	13.0 ± 1.1 (10)
L-[U-^{14}C]leucine (10.0)	29.2 ± 6.7 (10)	35.3 ± 4.9 (9)

enhances insulin release evoked by D-glucose, L-leucine or the association of Ba^{2+} and theophylline. Since *N-p*-tosylglycine did not stimulate insulin release in the absence of another secretagogue, the secretory response to *N-p*-tosylglycine could be looked upon as a facilitation, rather than initiation, of insulin release.

The facilitating action of *N-p*-tosylglycine upon insulin release was apparently not attributable to a nutrient-like effect of the drug. Indeed, *N-p*-tosylglycine failed to affect the oxidation of D-glucose or L-leucine. *N-p*-tosylglycine also failed to mimic the stimulant action of nutrient secretagogues upon ^{45}Ca net uptake [11]. Moreover, glycine did not reproduce the insulinotropic effect of *N-p*-tosylglycine.

A primary effect of *N-p*-tosylglycine upon ionic fluxes also appears as an unlikely explanation for its facilitating action upon insulin release. Thus, at variance with the situation found in response to secretagogues such as hypoglycaemic sulphonylureas [16], *N-p*-tosylglycine failed to provoke any obvious change in ^{45}Ca outflow from prelabelled and perifused islets.

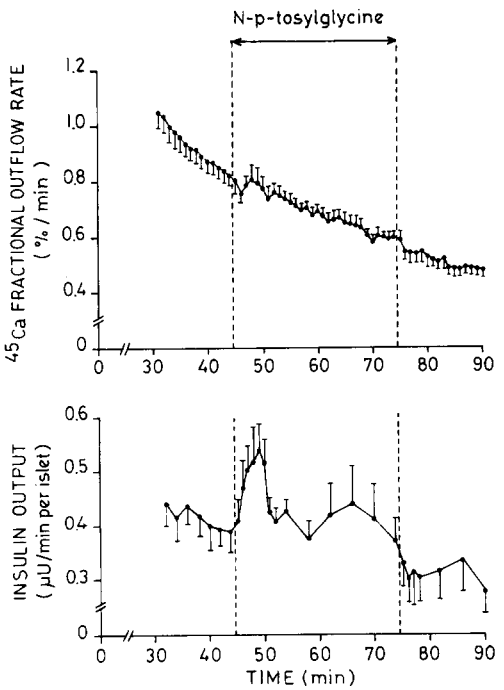

Fig. 1. Effect of *N-p*-tosylglycine (5 mM) upon ^{45}Ca fractional outflow rate and insulin release by islets perifused at normal Ca^{2+} concentration (1.0 mM) in the presence of 7.0 mM glucose. Mean values (±S.E.M.) for ^{45}Ca outflow and insulin release refer to the same six individual experiments.

Two series of observations argue against the view that *N-p*-tosylglycine affected the activity of transglutaminase within the islet cells. First, *N-p*-tosylglycine was still able to augment insulin release in islets exposed to methylamine, although the latter drug is known to penetrate into islet cells and to inhibit transglutaminase activity in intact islets [3, 4]. Second, in intact islets, *N-p*-tosylglycine failed to affect the incorporation of [2,5-^{3}H]histamine into cellular proteins.

Since *N-p*-tosylglycine inhibited transglutaminase activity in islet homogenates, but apparently failed to do so in intact islets, the question comes to mind whether *N-p*-tosylglycine penetrates into the islet cells. No radioactive *N-p*-tosylglycine was readily available to investigate this question. Since the esterification of certain compounds may facilitate their entry into cells, we have tested the effect of the methyl ester of two *Nα-p*-tosylated amino acids, which were previously reported to affect transglutaminase activity [17]. Neither of these methyl esters reproduced the facilitating action of *N-p*-tosylglycine upon insulin release.

Our results strongly suggest that *N-p*-tosylglycine acted in the secretory sequence upon some late event, at a site distal to the provision of Ca^{2+}. For instance, *N-p*-tosylglycine could interfere with secretory events at the cell boundary [18]. This could explain why the drug failed to enhance insulin release in the presence of hypoglycaemic sulphonylureas, which may also act at the same level and, incidentally, also affect transglutaminase activity in islet homogenates [17]. The proposed interference with a late event in the secretory sequence could account for the fact that *N-p*-tosylglycine facilitated insulin release evoked by both nutrient (D-glucose, L-leucine) and nonnutrient (Ba^{2+}) secretagogues. It would also account for the fact that the facilitating action of *N-p*-tosylglycine upon insulin release represented an immediate phenomenon. Last, since *N-p*-tosylglycine exerted little or no effect upon the elevated rate of insulin release evoked by a high concentration of glucose or the association of L-leucine and L-glutamine, it is conceivable that the potent activation of the secretory process by these nutrients involves stimulation of membrane-associ-

ated events analogous to those otherwise facilitated by *N*-*p*-tosylglycine [2]. The nature of the events affected by *N*-*p*-tosylglycine is open to speculation. It is interesting to note, however, that, in other tissues, transglutaminase is thought to catalyze the ε-(γ-glutamyl)lysine crosslinking of membrane proteins [19] and that *N*-*p*-tosylglycine may act as an activator, rather than inhibitor of certain transglutaminases [20].

In conclusion, the facilitating action of *N*-*p*-tosylglycine upon insulin release does not necessarily detract from the view that a Ca^{2+}-responsive transglutaminase participates in the mechanical events leading to or associated with the access of secretory granules to their site of exocytosis at the plasma membrane. Decisive factual evidence is nevertheless still required to document such a role for transglutaminase in the pancreatic B-cell.

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