Effects of 10 μM β-PTX and 10 μM δ-PTX on high affinity glutamate uptake in the rat hippocampal stratum radiatum of CA3

	Grain density (g/100 μm²) (SD) (n = 18)	% Inhibition in rat hippocampus	% Inhibition in insect nmj's[c]
Control	14.34 (2.77)		
β-PTX	11.73 (2.45)[a]	18%	0
δ-PTX	3.74 (1.45)[b]	74%	50–85%

[a] Difference from control: p = 0.05 (Student's t-test) [b] Difference from control: p = < 0.005 (Student's t-test) [c] nmj: neuromuscular junction, adapted from Storm-Mathisen[4].

Krebs solution containing either 10 μM β-PTX or 10 μM δ-PTX, for 30 min. Subsequently, during 10 min, the slices were incubated in Krebs solution containing toxin (either 10 μM β-PTX or 10 μM δ-PTX) with the addition of 2.3 μM (50 μCi/ml) ^{3}H glutamate. After incubation the slices were rinsed in Krebs solution (2 × 10 min), fixed in glutaraldehyde (5%), dehydrated and embedded in epon. Control slices were preincubated and incubated as described, not including the addition of the toxin. Preincubation and incubation were performed at 25°C. Autoradiographs were prepared from serial sections (2 μm) through the hippocampal slices, using Kodak Ntb_2 liquid emulsion. After an exposure of 6 days the autoradiographs were developed and stained through the emulsion with toluidine blue.

Results and discussion. The hippocampal formation was used for the investigation of the effects of β-PTX and δ-PTX on the vertebrate central nervous system, since it is a much used and thoroughly investigated cerebral structure with an extensive glutamatergic system[3,4].

It has been shown in the literature that the labeling of hippocampal slices incubated under the conditions described above (2.3 μM (50 μCi/ml) ^{3}H glutamate in Krebs, 10 min) is abolished if sodium ions are omitted from the incubation medium or if aspartate is present in the incubation medium[7,9]. This indicates that high affinity glutamate uptake is responsible for the ^{3}H glutamate accumulated in the hippocampus and that no receptor-bound ^{3}H glutamate contributes to the labeling. Moreover, incubation conditions for the demonstration of receptor-bound ^{3}H glutamate are completely different[10]. The possibility of a low affinity uptake system contributing to the accumulated ^{3}H glutamate may be neglected, considering the low concentration of ^{3}H glutamate in the medium (2.3 μM, 50 μCi/ml) and the short incubation time (10 min). Therefore this established and acknowledged method was adopted for the present investigation of the effects of the two polyamine insect venom compounds on high affinity glutamate uptake.

To exclude erroneous results owing to slow penetration of either ^{3}H glutamate or the toxins, grain counts were made above the stratum radiatum of the CA_3 region from serial sections through both control and toxin treated hippocampal slices (200 μm). The aforementioned counts revealed that the grain counts from serial sections through one hippocampal slice were identical. This holds for control slices as well as for toxin-treated slices. The results of this investigation were interpreted as an indication that the penetration of both glutamate and the two toxins into the slices was sufficient and that neither the amount of glutamate nor the amount of either of the two toxins were limiting factors. The grain counts in the 2-μm sections above the stratum radiatum of the CA3 region revealed that both β-PTX and δ-PTX significantly reduce the amount of accumulated glutamate (table). The greatest reduction of high affinity glutamate uptake (74%) was observed in slices treated with δ-PTX. A small, but still significant reduction (18%) was observed in slices treated with β-PTX. The 74% reduction of glutamate owing to the action of 10 μM δ-PTX is comparable to the inhibition observed in the glia and terminal axons of insect neuromuscular junctions[2]. However, in insect neuromuscular junctions no effect of β-PTX can be observed, whereas in hippocampal slices β-PTX reduces the glutamate uptake significantly (18%).

The above observations indicate that the polyamine-like δ-PTX and possibly β-PTX, both of which affect the glutamatergic transmission in insects[1], are also active in the glutamatergic transmission in rat brain. The action of these toxins with their polyamine structure is in agreement with the observation that polyamines act as specific uptake inhibitors in synaptosome preparations[11].

Hence these toxins and their structural analogues may serve as pharmacological tools for the investigation of glutamatergic transmission processes, as it was possible to use natural toxins for the elucidation of cholinergic transmission processes[12].

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0014-4754/86/020157-02$1.50 + 0.20/0

The role of the autonomic nervous system in mediating pancreatic endocrine responses to arginine in the calf[1]

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Summary. The release of insulin which occurred in response to arginine, in the conscious calf, differed from that which occurs in response to glucose in that it was not significantly affected by either adrenergic or muscarinic blocking agents. Release of pancreatic glucagon was reduced by pretreatment with phentolamine.
Key words. Arginine; endocrine pancreas; autonomic nervous system.

The release of insulin from the pancreas which normally occurs in response to hyperglycemia in the conscious calf is mediated largely via the parasympathetic innervation[2]. In addition, it is effectively abolished by pretreatment with propranolol alone (0.25 mg/kg i.v. 10 min before the glucose challenge) but not by combined pretreatment with both propranolol and phentol-

amine[3], which suggests that insulin release is continually influenced by tonic α-adrenergic inhibition[4] and that this is normally roughly matched by tonic β-adrenergic excitation[5]. The present study was designed to assess the role of the autonomic innervation in mediating pancreatic endocrine responses to arginine, which is another potent insulinotropic agent, but also stimulates the release of pancreatic glucagon and so has been used to test islet function clinically[6].

Methods. The experiments were carried out in conscious pedigree Jersey calves 3–6 weeks after birth. Preparatory surgery involved the insertion of a narrow-bore catheters into the abdominal aorta via the saphenous arteries under general anesthesia together with section of the splanchnic nerves in some animals. Just before each experiment a braunula cannula was inserted into a jugular vein under local anesthesia to provide a conduit for i.v. injections or infusions. Autonomic blockade was achieved by the i.v. administration of one or more of the following agents 10 min prior to starting an infusion of arginine: atropine (atropine sulphate, BDH, 0.2 mg/kg), propranolol (Inderal, ICI, 0.25 mg/kg), phentolamine (Rogitine, CIBA, 0.1 mg/kg + 0.02 mg·kg^{-1}·min^{-1}). These doses were employed because they have been found previously to be supramaximal for pancreatic neuroendocrine responses to glucose[7]. Arginine (l-arginine hydrochloride, Sigma) was dissolved in sterile physiological saline and infused at a dose of 0.02 mmol·kg^{-1}·min^{-1} for 30 min in a volume of 2.5 ml/min. Samples of arterial blood were collected at intervals into heparinized tubes containing aprotinin (Trasylol, Bayer,1000 KIU/ml blood) and centrifuged without delay at +4 °C; the plasma was subsequently stored at −20 °C. Glucose was estimated by means of a Mark 2 Beckman Glucose Analyzer. Pancreatic glucagon, insulin and pancreatic polypeptide (PP) were measured by radioimmunoassays as described previously[7]. Statistical analyses were made according to the methods of Snedecor and Cochran[8].

Results and discussion. An i.v. infusion of arginine (0.02 mmol·kg^{-1}·min^{-1} for 30 min) was found to produce a substantial rise in the mean concentration of both pancreatic glucagon and insulin in the arterial plasma of normal conscious calves (fig. 1). These changes were associated with an initial rise in mean plasma glucose concentration followed by a fall (fig. 1) and this delayed hypoglycemia was associated with a substantial rise in mean plasma PP concentration (fig. 2). Closely similar changes in mean plasma glucagon and insulin concentration occurred in response to the same dose of arginine in calves with cut splanchnic nerves which were pretreated with atropine but the rise in mean plasma glucose concentration was significantly less than that in the control group ($p < 0.02$). Thus, the average mean arterial plasma concentrations during the infusion in the control group were glucose 1.03 ± 0.08 mmol/l, glucagon 58 ± 6 pmoles/l and insulin 1954 ± 349 pmoles/l. The corresponding values in the atropinized calves with cut splanchnic nerves were glucose 0.23 ± 0.22 mmol/l, glucagon 75 ± 13 pmoles/l and insulin 1448 ± 143 pmoles/l. In contrast, the delayed rise in mean plasma PP was completely abolished. It was subsequently established that this was due to muscarinic blockade with atropine rather than to section of the splanchnic nerves, by examining the effects of exogenous arginine in calves which had either had the splanchnic nerves sectioned or been pretreated with atropine but had intact splanchnic nerves. This confirms previous findings that PP is released in response to hypoglycemia and that the effect is mediated via the parasympathetic innervation[9].

In spite of the fact that bilateral section of the splanchnic nerves failed to affect any of the pancreatic endocrine responses significantly, the effect of pretreatment with either propranolol or phentolamine or both was investigated in case adrenergic effects were counteracted by peptides which might be released from autonomic nerve terminals. The rise in mean plasma insulin concentration was unaffected by either blocking agent or by a combination of the two (table and fig. 2); the average mean plasma insulin concentration during the infusion was

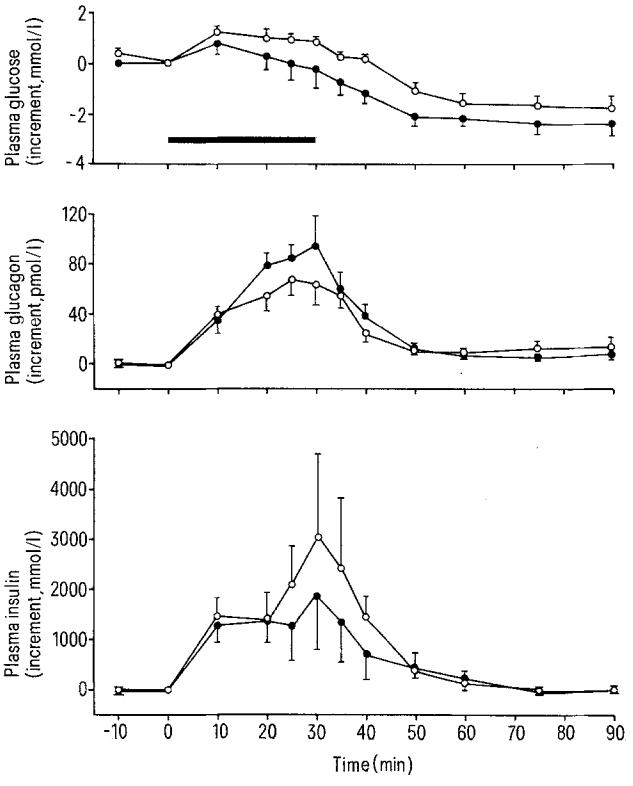

Figure 1. Comparison of the changes in mean plasma glucose, pancreatic glucagon and insulin concentration in response to i.v. arginine (0.02 mmol·kg^{-1}·min^{-1} for 30 min) in normal conscious 3–6-week-old calves (○; n = 9) and in calves with cut splanchnic nerves pretreated with atropine (0.2 mg/kg; ●; n = 7). Vertical bars: SE of each mean value. Horizontal bar: duration of infusion. Absolute values at time = 0 in the normal calves: glucose 3.7 ± 0.3 mmol/l, glucagon 6 ± 1 pmol/l, insulin 46 ± 27 pmol/l. Calves with cut splanchnic nerves given atropine: glucose 3.8 ± 0.5 mmol/l, glucagon 3 ± 1 mmol/l, insulin 13 ± 3 pmol/l.

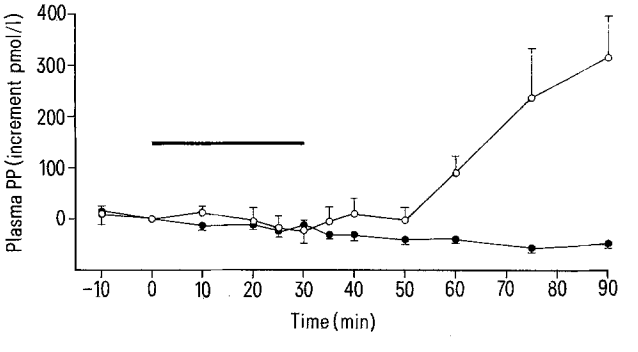

Figure 2. Comparison of the changes in mean plasma pancreatic polypeptide concentration in response to i.v. arginine (0.02 mmol·kg^{-1}·min^{-1} for 30 min) in normal conscious 3–6-week-old calves (○; n = 9) and in calves with cut splanchnic nerves pretreated with atropine (0.2 mg/kg; ●; n = 9). Vertical bars: SE of each mean value. Horizontal bar: duration of infusion.

Comparison of the incremental changes in mean arterial plasma glucose, pancreatic glucagon, insulin and PP in normal conscious 3–6-week-old calves pretreated with either propranolol (0.25 mg/kg, n = 6) or phentolamine (0.1 mg/kg + 0.02 mg · kg^{-1} · min^{-1}, n = 6) during an i.v. infusion of arginine (0.02 mmol · kg^{-1} · min^{-1} for 30 min)

Infusion (min)	Glucose (mol/l) Propranolol	Phentolamine	Glucagon (pmol/l) Propranolol	Phentolamine	Insulin (pmol/l) Propranolol	Phentolamine	PP (pmol/l) Propranolol	Phentolamine
10	0.5 ± 0.4	0.32 ± 0.4	30 ± 8	17 ± 9	2229 ± 739	1235 ± 1282	62 ± 80	111 ± 73
20	− 0.23 ± 0.4	0.23 ± 0.5	49 ± 19	23 ± 14	1780 ± 1057	736 ± 297	52 ± 76	262 ± 240
25	− 0.33 ± 0.4	− 0.15 ± 0.7	47 ± 23	24 ± 16	548 ± 166	1269 ± 512	71 ± 53	159 ± 116
30	− 0.7 ± 0.2	− 0.2 ± 0.9	41 ± 20	27 ± 20	542 ± 192	1522 ± 559	150 ± 110	44 ± 46
Absolute mean values at time = 0								
	3.3 ± 0.3	4.2 ± 0.5	9 ± 5	6 ± 1	102 ± 78	23 ± 2	120 ± 58	122 ± 45

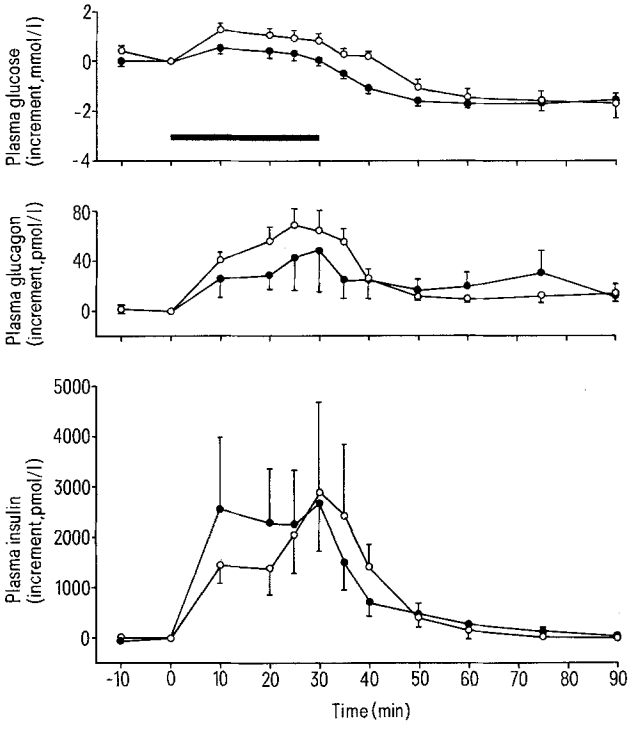

Figure 3. Comparison of the changes in mean plasma glucose, pancreatic glucagon and insulin concentration in response to i.v. arginine (0.02 mmol·kg^{-1}·min^{-1} for 30 min) in normal conscious 3–6-week-old calves (○, n = 9) and in calves pretreated with propranolol and phentolamine (0.25 mg/kg and 0.1 mg/kg + 0.02 mg·kg^{-1}·min^{-1}, ●, n = 7). Vertical bars: SE of each mean value. Horizontal bar: duration of infusion. Absolute values at time = 0 in the normal calves: glucose 3.7 ± 0.3 mmol/l, glucagon 6 ± 1 pmol/l, insulin 46 ± 27 pmol/l. In the calves given propranolol and phentolamine: glucose 3.8 ± 0.2 mmol/l, glucagon 5 ± 1 pmol/l, insulin 39 ± 23 pmol/l.

2454 ± 108 pmoles/l in the group given both phentolamine and propranolol, compared with a value of 1954 ± 349 pmoles/l in the control group. However, the average rise in mean plasma glucagon concentration which occurred in response to arginine (58 ± 6 pmoles/l) was significantly reduced by combined α- and β-adrenoceptor blockade (37 ± 6 pmoles/l, $p < 0.05$), as was the rise in mean plasma glucose concentration (from 1.03 ± 0.08 mmol/l to 0.3 ± 0.1 mmol/l). Administration of propranolol and phentolamine separately showed that this was due to α-adrenoceptor blockade (table).

There was a delayed rise in mean plasma PP concentration in each of these groups of animals indicating that this response was certainly not mediated via adrenergic receptors, although it might possibly have been partially inhibited thereby (fig. 4).

The importance of the autonomic innervation to the pancreas in mediating endocrine responses to glucose and other stimuli has been the subject of recent reviews[10,11]. The results of the present

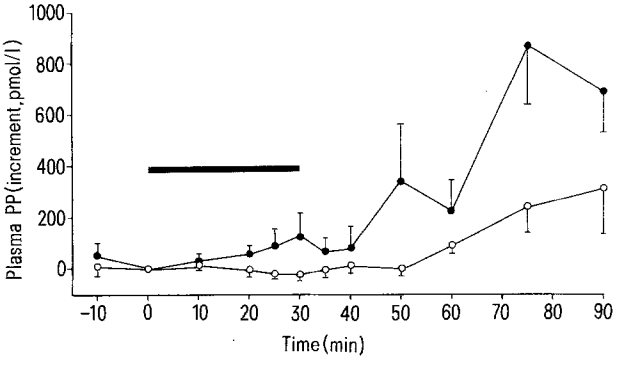

Figure 4. Comparison of the changes in mean plasma pancreatic polypeptide in response to i.v. arginine (0.02 mmol·kg^{-1}·min^{-1} for 30 min) in normal conscious 3–6-week-old calves (○, n = 9) and in calves pretreated with propranolol and phentolamine (0.25 mg/kg and 0.1 + 0.02 mg·kg^{-1}·min^{-1}, ●, n = 7). Vertical bars: SE of each mean value. Horizontal bar: duration of infusion.

study in the calf show that the release of insulin which occurs in response to arginine differs from these in so far as it is not mediated via the autonomic innervation. In contast, α-adrenoceptors do seem to contribute to the release of pancreatic glucagon which arginine provokes. The fact that arginine induces insulin release by a direct action on the β-cells of the islets in vivo, whereas glucose acts principally via the innervation, needs to be borne in mind whenever it is employed to evaluate islet function.

1 This work was supported by the British Diabetic Association. It is a particular pleasure to acknowledge the skilled assistance provided by Messrs P. M. M. Bircham and G. P. McGregor.
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0014-4754/86/020158-03$1.50 + 0.20/0