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Effect of *n*-hexacosanol on insulin secretion in the rat

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Abstract

n-Hexacosanol, a long-chain satured fatty alcohol extracted from *Hygrophyla erecta Hochr.*, has been recently shown to exert neurotrophic properties on central neurons and to stimulate phagocytosis in macrophages. The present work was designed to investigate the effects of hexacosanol on stimulated insulin secretion in vivo and in vitro. In anaesthetized rats, hexacosanol (2 mg/kg i.p.) induced a reduction of the insulin response to an intravenous glucose tolerance test (0.3 g/kg) with a consequent increase in hyperglycaemia. In vitro, in the isolated perfused pancreas, hexacosanol at the concentration of 10^{-7} M clearly reduced the two phases of glucose-induced insulin secretion. At the higher concentration (10^{-5} M), hexacosanol was no longer able to exert an inhibition of glucose-induced insulin release; surprisingly a stimulating effect occurred which was of the same magnitude as in control experiments with Tween alone, at the concentration used to dissolve hexacosanol. In isolated perifused islets, 22 mM glucose-stimulated insulin release was also inhibited by hexacosanol at the concentrations of 10^{-9} M and 10^{-7} M, but not at 10^{-5} M. In contrast, insulin secretion induced by arginine (20 mM) was not affected by the different concentrations of hexacosanol. It is concluded that *n*-hexacosanol at 10^{-9} M and 10^{-7} M exerts an inhibitory effect on insulin secretion stimulated by glucose in vivo and in vitro in the rat, suggesting a direct effect on islets of Langerhans.

Keywords: Fatty alcohol; Insulin secretion; Islet of Langerhans; Pancreas

1. Introduction

n-Hexacosanol is a long-chain fatty alcohol (C26:0) isolated from a tropical plant, *Hygrophyla erecta Hochr.* (*Acanthaceae*), reported to be used in Vietnam and India to cure wounds (Pletelot, 1953). More recently, this compound has been shown to stimulate phagocytosis in macrophages (Moosbrugger et al., 1992) and to induce marked biochemical and morphological changes in neurons maintained in culture, suggesting that it affects their differentiation (Borg et al., 1987). *n*-Hexacosanol also improves neuronal survival in vivo following axotomy in the brain or the injection of the neurotoxic compound kainic acid (Borg et al., 1990; Borg, 1991), suggesting it has a trophic action. Thus, it might be speculated that *n*-hexacosanol could also display a trophic effect on peripheral neurons, and consequently could act against peripheral neuropathies, e.g. those known to occur during the diabetic state. Insulin is considered as a survival factor for neurons (Wozniak et al., 1993) and thus it was of interest to determine whether *n*-hexacosanol exerts an effect on insulin secretion itself.

For this purpose, we were interested to investigate whether *n*-hexacosanol interferes with pancreatic endocrine function in the rat, using in vivo and in vitro experimental models.

2. Materials and methods

Fed male Wistar rats were used in all experiments.

2.1. In vivo experiments

Rats weighing 400–450 g were anaesthetized with an intraperitoneal injection of sodium pentobarbitone (60

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mg/kg) and placed on a heated operating surface. A catheter, filled with heparin-saline to avoid blood clotting, was inserted into a jugular vein. Blood samples were taken from the catheter. In all experiments, after a 30 min resting period, three samples (0.5 ml over 1 min) were taken to determine basal plasma insulin and glucose levels.

In these conditions, the effects of *n*-hexacosanol on an intravenous glucose tolerance test were studied. For this purpose, *n*-hexacosanol diluted in 10% Tween 80 was intraperitoneally injected at a dose of 2 mg/kg, 5 min before the intravenous glucose injection (0.3 g/kg) for 1 min. Control experiments were performed with an intraperitoneal injection of the same quantity of 10% Tween 5 min before intravenous glucose.

2.2. In vitro experiments

Experiments on the isolated perfused pancreas

The surgical procedure for the isolated rat perfused pancreas has been described previously (Loubatières et al., 1969; Bertrand et al., 1986). After the rats (330–350 g) had been anaesthetized with sodium pentobarbitone (60 mg/kg i.p.), the pancreas was totally isolated from all neighbouring tissues. The pancreas was perfused through its arterial system with a Krebs-Ringer bicarbonated buffer containing pure bovine serum albumin (2 g/l). A mixture of O_2 (95%) and CO_2 (5%) was continuously bubbled through this medium; the pH was 7.4. The preparation was maintained at 37.5°C.

In all experiments, basal glucose concentration was 5 mM. A 30 min adaptation period was allowed before the first sample was taken. Then two more samples were taken at 40 and 45 min. In the presence of 5 mM glucose, *n*-hexacosanol was perfused alone from time 45 to 60 min. At time 60 min, the glucose concentration was raised from 5 mM to 11 mM. This latter concentration was maintained up to 80 min, when the glucose concentration was returned to 5 mM. *n*-Hexacosanol was perfused at 10^{-5} or 10^{-7} M. Control experiments were performed with or without Tween 80 alone which was used to dissolve *n*-hexacosanol, its final concentration being 8×10^{-3} and 8×10^{-5}% for 10^{-5} and 10^{-7} M hexacosanol respectively. The flow rate was measured for 1 min for each sample. Samples were immediately frozen for insulin evaluations.

Experiments on isolated and perifused islets of Langerhans

Islets of Langerhans were isolated after collagenase digestion of the pancreas, according to a technique derived from that of Lacy and Kostianovsky (1967). Immediately after isolation, the islets were perifused for 90 min at 37.5°C in a Krebs-Ringer bicarbonate buffer, pH 7.4, containing 1 g/l bovine serum albumin.

Batches of 25 islets were then perifused in parallel chambers using a system similar to that described by Henquin (1979). The basal glucose concentration was 3 mM for 30 min. Then insulin release was stimulated by glucose (22 mM) or by arginine (20 mM) for the following 30 min and the glucose concentration was returned to basal values (3 mM) for the last 30 min.

n-Hexacosanol was infused at different concentrations (10^{-5}, 10^{-7} and 10^{-9} M) from 15 to 60 min. It was diluted in Tween 80 with a final concentration of 8×10^{-3}, 8×10^{-5} and 8×10^{-7}% for the respective hexacosanol concentrations.

Control experiments were performed with glucose or arginine alone.

The flow rate was 1.4 ml/min. Samples from the perfusate, collected at 5 min intervals, were immediately frozen for insulin determinations. After the last sampling, islets were incubated for 24 h in acid ethanol (pure HCl and 75% ethanol, 1:65 v/v) for determination of islet insulin content.

2.3. Assays

Blood glucose was measured using a Technicon autoanalyser with the potassium ferricyanide method (Alric et al., 1965). Insulin concentration was measured by the method of Herbert et al. (1965), using an antibody supplied by Miles Laboratories (Paris, France). [^{125}I]Insulin was obtained from International CIS (Gif-sur-Yvette, France); the standard used was rat insulin (Novo, Copenhagen, Denmark) whose biological activity was 22.3 μU/ng. The intra- and inter-assay coefficients of variations were 9% and 13.5% respectively.

2.4. Expression of data and statistical analysis

Data are expressed as means ± S.E.M. All data were submitted to an analysis of variance followed by the multiple comparison test (Zar, 1974). For glucose tolerance tests, the areas under the curves (AUCs) were calculated.

2.5. Drugs

n-Hexacosanol was obtained from Medafor (67410-Illkirch-Graffenstaden, France). Collagenase was supplied by Serva (Heidelberg, Germany) and Tween 80 was from Sigma Chemical Company (St. Louis, MO, USA).

3. Results

3.1. In vivo experiments

In anaesthetized rats, we tested the in vivo effect of *n*-hexacosanol on the intravenous glucose tolerance

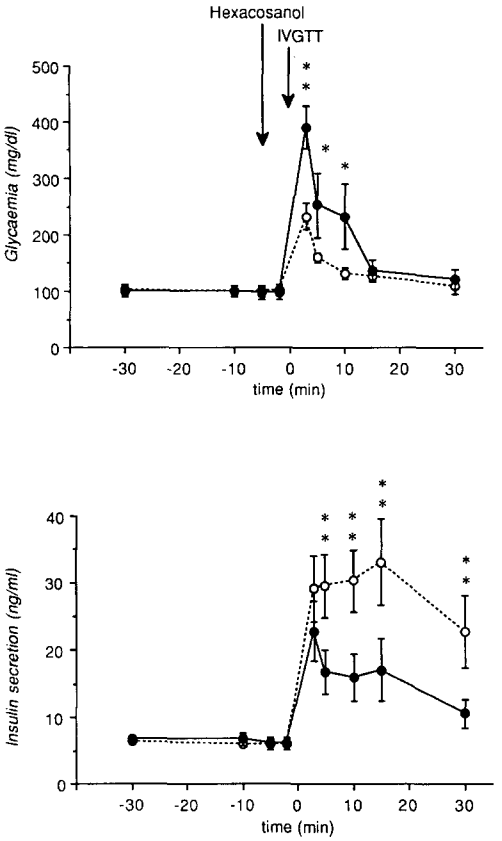


Fig. 1. Effects of *n*-hexacosanol (2 mg/kg intraperitoneally) on the increases in plasma insulin and glucose levels in response to intravenous glucose injection (IVGTT) (0.3 g/kg) in anaesthetized rats (●). Control animals received intravenous glucose injection alone (○). Data are means ± S.E.M. of 7 experiments. * $P < 0.05$, ** $P < 0.01$.

test (Fig. 1). In control experiments, glucose injection (0.3 g/kg) induced an increase in glycaemia; the blood glucose concentration at the third minute reached 232.6 ± 28.4 mg/dl. It then progressively returned to basal values at 30 min. The rise in glucose levels was associated with an increase in plasma insulin concentrations, which rose to 29.2 ± 4.9 ng/ml at 3 min and remained high up to 30 min.

When *n*-hexacosanol (2 mg/kg) was injected before the intravenous glucose tolerance test, the increase in plasma insulin levels was significantly lower at 5, 10, 15 and 30 min ($P < 0.01$). The integrated insulin response (AUC) during the 30 min was 455 ± 102 ng/ml versus 838 ± 136 ng/ml in controls ($P < 0.01$). The maximum hyperglycaemia was higher (390.7 ± 46.3 mg/dl at 3 min, $P < 0.01$) and the return to basal values was less rapid than in controls. The integrated glucose response was higher than in controls (5462 ± 540 mg/dl versus 4045 ± 294 mg/dl, $P < 0.01$).

When *n*-hexacosanol (2 mg/kg) was administered chronically in rats (once a day for 20 successive days), blood glucose concentration in response to an intravenous glucose tolerance test performed on day 21 was quite similar to that of control animals.

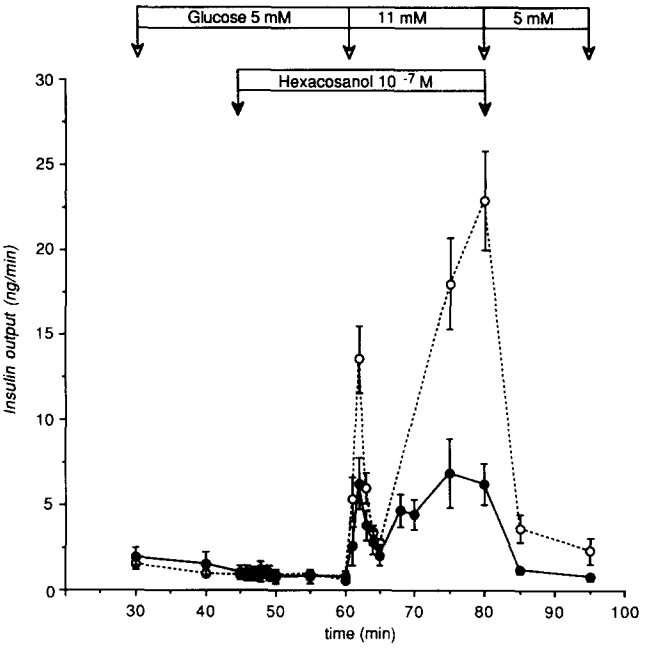


Fig. 2. Effects of *n*-hexacosanol (10^{-7} M) on insulin secretion induced by a rise in glucose concentration (5 to 11 mM) in isolated perfused rat pancreas (●). Control pancreas received glucose alone (○). Data are means ± S.E.M. of 5 to 8 experiments.

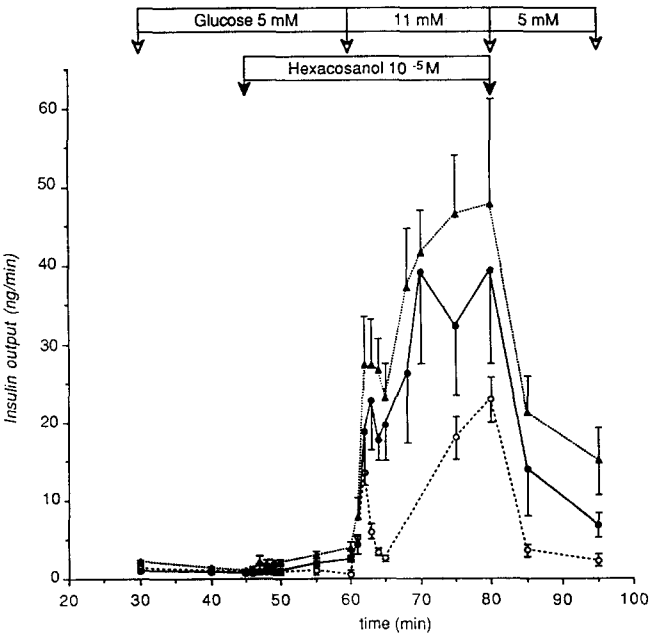
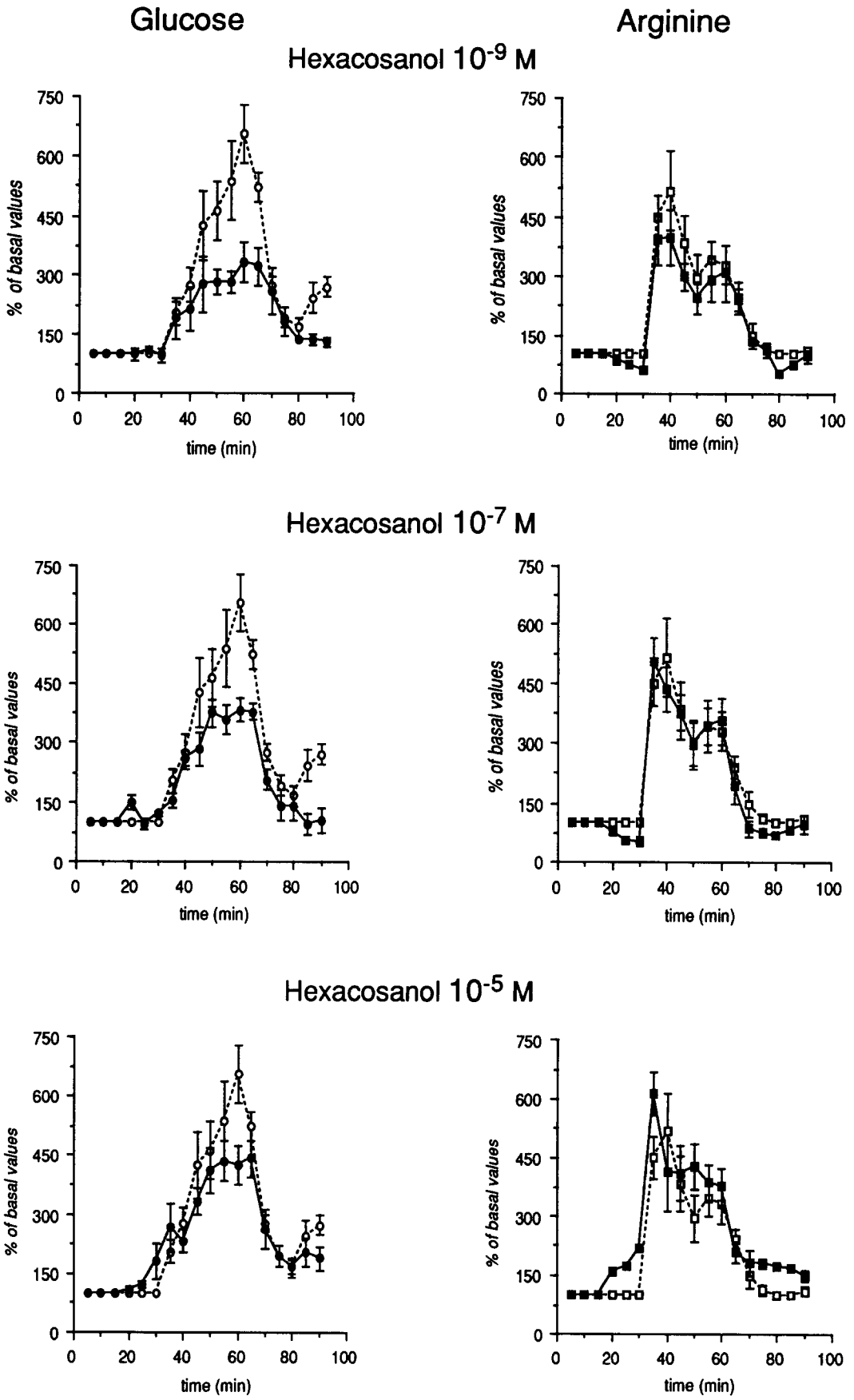


Fig. 3. In isolated rat pancreas, effects on insulin secretion induced by a rise in glucose concentration (5 to 11 mM) of *n*-hexacosanol (10^{-5} M) dissolved in Tween 80 (●) or of the same quantity of Tween 80 (▲). Control pancreas received glucose alone (○). Data are means ± S.E.M. of 5 to 6 experiments.


Glucose
Arginine
Hexacosanol 10-9 M
Hexacosanol 10-7 M
Hexacosanol 10-5 M
% of basal values
time (min)
0
150
300
450
600
750
0
20
40
60
80
100

3.2. In vitro experiments

On the isolated pancreas

The effects of *n*-hexacosanol on insulin secretion are illustrated in Figs. 2 and 3.

In the presence of the basal 5 mM glucose concentration, *n*-hexacosanol (10^{-7} M) dissolved in Tween 80 at 8×10^{-5}% did not influence insulin output. In control experiments, raising the glucose concentration in the perfusate from 5 mM to 11 mM elicited the classical biphasic pattern of insulin release. Administration of hexacosanol (10^{-7} M), 15 min before and during high glucose, markedly inhibited both phases of the insulin response. The AUC for the first phase (5 min) was 15.2 ± 4.4 ng versus 30.9 ± 4.2 ng in controls ($P < 0.05$) and for the second phase (20 min) 80.2 ± 22.6 ng versus 223.5 ± 30.3 ng in controls ($P < 0.01$). Moreover, the Tween 80 concentration used to dissolve 10^{-7} M hexacosanol did not modify the profile of insulin release.

Hexacosanol 10^{-5} M dissolved in Tween 80 at 8×10^{-3}% and Tween 80 alone at the same concentration were able to induce a similar slight and progressive increase in basal insulin output from 0.71 ± 0.10 ng/min to 2.48 ± 0.44 ng/min ($P < 0.001$) and from 1.22 ± 0.19 ng/min to 3.81 ± 0.90 ng/min ($P < 0.01$) respectively at 60 min. In the same way, hexacosanol at 10^{-5} M no longer inhibited glucose (11 mM)-induced insulin secretion; in contrast, a stimulating effect occurred that could also be observed, and with the same magnitude, in experiments with Tween 80 (8×10^{-3}%) alone. Thus, for the first phase, AUCs were 112.3 ± 18.8 ng and 83.1 ± 19.4 ng for Tween 80 and *n*-hexacosanol dissolved in Tween 80 respectively versus 30.9 ± 4.2 ng in controls ($P < 0.01$) and for the second phase 624.8 ± 89.2 ng and 481.4 ± 133 respectively versus 206.1 ± 28.1 ng in controls ($P < 0.01$).

The effects of *n*-hexacosanol on pancreatic flow rate varied according to the different concentrations of hexacosanol or Tween 80 used. At the concentration of 10^{-7} M, *n*-hexacosanol did not significantly modify pancreatic flow. In contrast at 10^{-5} M, *n*-hexacosanol provoked a clear decrease in pancreatic flow. This inhibitory effect was progressive: from 2.53 ± 0.02 ml/min (45 min) it reached 2.04 ± 0.11 ml/min at 60 min (−19%, $P < 0.01$). The pancreatic flow remained low throughout the *n*-hexacosanol perfusion. Tween 80 alone, at the concentration used to dissolve *n*-hexacosanol at 10^{-7} M, was ineffective but at the higher concentration used to dissolve *n*-hexacosanol at 10^{-5} M, a progressive decrease in pancreatic flow, from 2.52 ± 0.02 ml/min (45 min) to 2.12 ± 0.13 ml/min (−16%, $P < 0.05$), occurred. A similar pattern was obtained with *n*-hexacosanol at 10^{-5} M.

On isolated perifused islets of Langerhans

The effects of *n*-hexacosanol at the concentrations of 10^{-9}, 10^{-7} and 10^{-5} M on glucose (22 mM)- or arginine (20 mM)-stimulated insulin release by perifused islets of Langerhans are illustrated in Fig. 4.

Addition of 10^{-9} or 10^{-7} M *n*-hexacosanol caused a clear reduction in glucose-induced insulin release. Glucose alone induced an insulin release of 550% over the basal level. With *n*-hexacosanol 10^{-9} and 10^{-7} M, the peak reached +200% (−64%, $P < 0.01$) and +250% (−55%, $P < 0.01$) respectively. Only a slight inhibitory effect was noted with *n*-hexacosanol 10^{-5} M at time 60 min.

Arginine alone induced a stimulation of insulin release of 400%. Addition of *n*-hexacosanol at the different concentrations used (10^{-9}, 10^{-7}, 10^{-5} M) did not significantly modify this insulin release.

Concerning the insulin content of islets, no significant modification versus controls was observed with the different concentrations of *n*-hexacosanol in the presence of 20 mM arginine. However, in the presence of 22 mM glucose, the islets content of insulin was increased by hexacosanol: +45% ($P < 0.05$) for 10^{-9} M, +54% for 10^{-7} M ($P < 0.01$) and +40% ($P < 0.05$) for 10^{-5} M. Indeed, the concentrations of insulin expressed as μU/100 islets were 286 ± 11 μU, 321 ± 21 μU and 293 ± 28 μU respectively versus 221 ± 21 μU in control experiments.

4. Discussion

This study clearly shows that *n*-hexacosanol exerts an inhibitory effect on insulin secretion stimulated by glucose in vivo and in vitro in the rat. However, this effect was only observed for the lowest concentrations and no longer occurred when insulin release was activated by another stimulating agent such as arginine.

Our in vivo experiments showed that *n*-hexacosanol injected intraperitoneally was able to rapidly decrease the insulin response to glucose injection and consequently to increase hyperglycaemia. This effect was fast in onset, suggesting an easy transfer of *n*-hexacosanol, dissolved in Tween 80, from the peritoneal cavity to the systemic compartment. The concentration of *n*-

Fig. 4. Effects of *n*-hexacosanol on insulin release from perifused islets. Glucose (22 mM) or arginine (20 mM) was perifused from 30 to 60 min in the absence (○□) and in the presence (●■) of hexacosanol administered from 15 to 60 min at the concentrations of 10^{-9} M, 10^{-7} M and 10^{-5} M. Results, expressed as means ± S.E.M. of 6 experiments, are percentages of basal values obtained during the first 15 min with glucose 3 mM alone.

hexacosanol we used (2 mg/kg) in our in vivo experiments was close to that chronically administered (1 mg/kg per day) in a recent study which investigated a possible role of this agent against neurotoxic damage of central neurons (Borg, 1991). Nevertheless, this inhibitory effect of *n*-hexacosanol on insulin secretion seems to be acute. Indeed, after a prolonged administration of hexacosanol (2 mg/kg per day for 20 successive days), this effect disappeared (data not shown), suggesting a tolerance to the drug.

Our in vitro experiments performed on isolated perfused pancreas but also on isolated perifused islets provide evidence for a direct pancreatic action of *n*-hexacosanol. In both types of experiments, 10^{-7} M hexacosanol significantly inhibited glucose-induced insulin release. This effect was even more pronounced with 10^{-9} M hexacosanol in perifused isolated islets of Langerhans. However, it was not found with the highest concentration of hexacosanol (10^{-5} M), neither in perifused islets nor in the isolated perfused pancreas. The difference in the effects observed with both preparations might result from the different glucose increments used to stimulate insulin secretion.

The mechanism by which hexacosanol exerts a direct effect on the endocrine pancreatic cells is unknown. However, the present results show that the effect is not related to a reduction of pancreatic outflow since the latter was not affected by *n*-hexacosanol at 10^{-7} M.

In addition, since the insulin content of perifused islets was not found to be reduced, but even increased in the glucose experiments, an inhibitory effect of *n*-hexacosanol on β cell response via an effect on insulin biosynthesis can be excluded. The effect of *n*-hexacosanol on insulin secretion by the endocrine pancreas may be related to a modification of membrane properties. As reported by Bruneau et al. (1987), the binding of insulin to cultured hepatoma cells can be influenced by the lipid environment. In addition, long-chain alcohols have been shown to influence membrane fluidity and membrane properties in platelets and nervous tissue (Kitagawa et al., 1985; Franks and Lieb, 1986).

The observation that Tween 80 was able to affect insulin secretion is interesting. In our experimental conditions, Tween 80 at the concentrations used to dissolve *n*-hexacosanol at 10^{-9} and 10^{-7} M (8×10^{-7}% and 8×10^{-5}% respectively) had no effect on insulin secretion. In contrast, the higher concentration of Tween 80 used to dissolve *n*-hexacosanol at the concentration of 10^{-5} M (8×10^{-3}%) provoked, in vitro in isolated pancreas, an increase in glucose-induced insulin secretion. This effect was observed in basal conditions (5 mM glucose) but also in stimulated conditions (glucose raising to 11 mM). A possible explanation could be that Tween 80 used at the higher concentration may facilitate glucose uptake into β cells according to the well-known permeabilizing and solubilizing properties of detergents (Stanford and Reynolds, 1976; Provow and Velicelebi, 1987). Thus the Tween 80 concentration used to dissolve 10^{-5} M hexacosanol could counteract the inhibitory effect of hexacosanol on insulin secretion.

Such a hypothesis remains, however, to be further investigated to assess if a possible modification of the glucose transport system might be sufficient to make it become a rate-limiting step for glucose metabolism (Hellman et al., 1971).

Finally, the inability of *n*-hexacosanol to affect arginine-induced insulin secretion strongly suggests that this compound does not primarily act on calcium influx into β cells, which is an essential step in glucose- and arginine-induced insulin release (Malaisse-Lagae et al., 1971).

In conclusion, *n*-hexacosanol exerts a direct inhibitory effect on the glucose-stimulated secretion of insulin by islets of Langerhans, which results in vivo in an increase in glycaemia. This effect no longer occurred when insulin release was activated by another stimulating agent such as arginine. However, it is not yet understood by which mechanism *n*-hexacosanol exerts this effect.

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