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Effect of Pinealectomy on Plasma Glucose, Insulin and Glucagon Levels in the Rat

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Summary

In an attempt to know the role of the pineal gland on glucose homeostasis, the blood plasma concentrations of glucose, insulin and glucagon under basal conditions or after the administration of nutrients were studied in the jugular vein of conscious pinealectomized (Pn), melatonin-treated pinealectomized (Pn + Mel) and control (C) rats. Glucose levels were smaller in C than in Pn rats, while immunoreactive insulin (IRI) concentrations were significantly greater in C than in Pn rats. Contrary to this, immunoreactive glucagon (IRG) levels were significantly greater in Pn than in C animals. Melatonin treatment of Pn rats induces an increase of IRI concentrations and a reduction in IRG levels. Similar changes were obtained when hormonal determinations were carried out in porta blood plasma. Although ether anesthesia increases circulating glucagon levels in the porta and cava veins, the qualitative changes of plasma insulin and glucagon in Pn and Pn + Mel were similar to those found in conscious rats. To determine the effects of nutrients on pancreatic hormone release, intravenous arginine or oral glucose were administered to the animals of the three experimental groups. In C rats, both glucose and IRI levels reached a peak 30 minutes after glucose ingestion, decreasing thereafter. However, in Pn rats a glucose intolerance was observed, with maximum glucose and insulin concentrations at 60 minutes, while in Pn + Mel animals, glucose and IRI concentrations were in between the data obtained with the other two groups. Furthermore, glucose ingestion induced a significant reduction of IRG levels in all the groups. Insulin response to intravenous arginine was smaller in Pn rats as compared with the other groups. Although basal IRG concentrations were greater in Pn animals, the hormone response to intravenous arginine was similar to that found in C rats. These results suggested that the pineal gland is necessary to maintain the circulating levels of insulin and glucagon and that the inversion of the insulin/glucagon ratio after pinealectomy could shift metabolic activities to a catabolic mode.

Key-Words: *Pinealectomy – Melatonin Treatment – Glucose Intolerance – Insulin Release – Glucagon Release*

Introduction

The pineal gland, a neuroendocrine transducer that appears to modify the activities of several other endocrine organs, seems also to modulate glucose homeostasis and especially insulin release by pancreatic B cells. These last two statements have been suggested on the basis of the data obtained in recent years, although the results presented are somewhat contradictory. In fact, different authors working with pinealectomized rats have described hypoglycemia and hyperinsulinemia (*Csaba* and *Barath* 1971; *Milcu, Nanu-Ionesco* and *Milcu* 1971; *Nanu-Ionesco* and *Ionesco* 1969; *Nanu-Ionesco* and *Marcean* 1970; *Gorray, Quay* and *Ewart* 1979), which has been explained as a consequence of increased blood levels of substances that antagonize the action of insulin on peripheral target tissues (*Milcu, Nanu-Ionesco* and *Milcu* 1971; *Gorray, Quay* and *Ewart* 1979). Other authors have reported that melatonin increases blood glucose levels in pigeons (*McKeown, Hohn* and *George* 1975) and reduces glucose-induced insulin secretion in both mice and rats, though had no effect on basal insulin levels and on glucose tolerance tests in vivo (*Bailey, Atkins* and *Matty* 1974). In addition, clinical evidence suggests that the pineal gland may be related to certain cases of insulin resistance. Several authors have reported in children a recessively inherited syndrome with pineal gland hyperplasia, skin changes, dental precocity and dysplasia, mild virilization and glucose intolerance associated to hyperinsulinemia and insulin resistance (*Mason* and *Sly* 1937; *Rabson* and *Mendenhall* 1956; *Wiedman, Spranger, Mogharei, Kubler, Tolkodorf, Bontemps, Drescher* and *Gunschers* 1968; *Barnes, Palumbo, Hayles* and *Folgar* 1974; *West, Lloyd* and *Turner* 1975), which has been related to a widespread disturbance in neuroendocrine control. These findings indicate that pineal gland products may play a role in carbohydrate metabolism through modifications on insulin release, which may be amplified under specific pathological situations. However, at present, insulin effects cannot be analyzed alone, because both insulin and glucagon are considered as components of a single bihormonal unit in which the counterbalancing and opposing biologic activities of both hormones upon the liver and other common target tissues have been well established (*Unger* and *Orci* 1976; *Unger* and *Orci* 1981).

Accordingly this study was designed in order to evaluate the role of the pineal gland on the circulating levels of insulin and glucagon under basal conditions and after the administration of physiological stimuli. For this purpose, in control, pinealectomized and melatonin-treated pinealectomized groups of rats, blood concentrations of glucose, insulin and glucagon were studied before and after the intravenous administration of arginine and of an oral glucose overload.

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Material and Methods

Experimental animals

Male Wistar rats weighing 200–250 g were housed under controlled conditions of light (12 hours alternating cycles of light and dark;

lights on from 8 a.m. to 8 p.m.) and temperature and were fed a standard diet (fat 3.8%, carbohydrates 49.5%, protein 21.4%) ad libitum. Three groups of experimental animals were used in this study: control (C), pinealectomized (Pn) and melatonin-treated pinealectomized (Pn + Mel) rats.

Surgical Procedures

Pinealectomy was carried out according to the procedure of *Perez, Casas, Villa Vigil* and *Bengoechea* (1979). Briefly, in rats anaesthetized with sodium pentobarbital (40 mg/kg body weight) a longitudinal cut was made 2 cm in front to 1 cm behind the inion, after which the epicraneal aponeurosis was cut and the skull deperiostized between both temporal lines. Using a cylindrical trepan powered by a dentist's drill a bone circle was cut centered on the lambda point, after which a section of the duramater was made, with the aid of an ophthalmic scalpel, along the lower edge of both lateral sinuses. Following this the right transverse sinus was cut in which blood circulation had previously been halted by electrocoagulation with a bipolar electrode. This procedure allowed us to remove the pineal gland and immediately to replace the duramater and the bone circle. With this surgical procedure the venous return was not subjected to any disturbances since blood was able to flow through the intact left sinus to the jugular vein; the survival rate of the animals according to this method was greater than 95%. After the surgical procedure, the animals were rested for 15 days, which was followed by another 15 days of melatonin treatment (250 µg/100 g body weight, injected subcutaneously) or by the administration of a placebo. Melatonin (Regis Chem. Co., USA) was dissolved in a minimum of absolute ethanol and diluted in a 0.9% NaCl solution. The placebo solution contained the same proportion of absolute ethanol and 0.9% NaCl. Unless otherwise indicated all glucose and hormonal determinations were carried out in the blood plasma from the jugular vein of conscious rats. When blood plasma samples were obtained from the porta and inferior cava veins, the rats were previously anaesthetized with ether. In order to obtain blood samples in conscious animals, one or two days before the experiment rats of different experimental groups were lightly anaesthetized with ether and a Silastic catheter was introduced into the jugular vein and anchored in place. In an attempt to maintain catheter permeability it was filled up with heparin. The rats recovered rapidly and were not fed overnight before intravenous arginine and oral glucose overload tests. To avoid anemia, each rat received its own red blood cells suspended in 0.9 percent saline. Arginine was administered intravenously as a bolus of 500 mg/kg body weight and oral glucose at a dose of 2 g/kg body weight was administered using a Teflon catheter introduced into the stomach.

Analytical procedures

Blood samples were collected in prechilled test tubes containing Trasylol (FBA Pharmaceuticals, New York, 500 KIU/ml) and EDTA (1.2 mg/ml) centrifuged at 4°C and the plasma was kept frozen until analyzed. Blood glucose was determined according to *Hugget* and *Nixon* (1975). Glucagon immunoreactivity (IRG) was measured as described previously (*Faloona* and *Unger* 1974) with a specific antiglucagon serum (30K) generously donated by Dr. R. Unger. This antiserum is considered to be reactive with the C-terminal portion of the glucagon molecule and crossreacts only very weakly with glucagon-like immunoreactivity (GLI). Insulin immunoreactivity (IRI) was measured by the method of *Herbert, Lau* and *Gottlieb* 1965), using rat insulin standards (Novo Laboratories, Copenhagen, Denmark) and guinea pig antiserum 607/9 against porcine insulin (a generous gift of Dr. P. Wright).

Statistical Analysis

Results were expressed as mean ± SEM. For statistical comparison Student's t-test was used.

Results

Basal blood plasma levels of glucose, insulin and glucagon in control, pinealectomized and melatonin-treated pinealectomized rats

Table 1 Basal concentrations of glucose, insulin and glucagon in blood plasma of control, pinealectomized and melatonin-treated pinealectomized rats. Means ± SEM. N = 23

Groups of rats	Plasma glucose (mg/dl	Plasma insulin (µU/ml)	Plasma glucagon (pg/ml)
Control	74± 5	34±5	188±23
Pinealectomized	100±11	20±3	291±42
Pinealectomized plus melatonin	95±15	30±4	227±43

Blood samples were obtained from the jugular vein in overnight-fasted conscious rats.

Table 2 Basal concentrations of insulin and glucagon in the blood plasma of the porta and inferior cava veins of control, pinealectomized and melatonin-treated pinealectomized rats. Means ± SEM. N = 14

Groups of rats	Plasma insulin (µU/ml)		Plasma glucagon (pg/ml)	
	PV	VC	PV	VC
Control	90±12	39±5	760± 94	407±48
Pinealectomized	71±13	31±6	1132±187	518±86
Pinealectomized plus melatonin	103±21	35±5	799± 86	386±49

Rats were lightly anaesthetized with ether.
PV: Porta vein. VC: Inferior vena cava.

To determine whether basal circulating levels of glucose, insulin and glucagon may be modified by pinealectomy, blood samples were collected from overnight-fasted conscious rats and those substances were measured according the procedures cited above. In pinealectomized (Pn) rats (Table 1) plasma glucose levels were greater than in control (C) animals ($P < 0.05$), while the values found in melatonin-treated pinealectomized rats (Pn + Mel) were intermediate between the other two groups. Contrariwise, IRI concentrations in C rats were significantly greater ($P < 0.05$) than in Pn animals. No significant differences ($P > 0.05$) between C and Pn + Mel animals were found. Also, plasma glucagon levels were seen to increase significantly ($P < 0.05$) after pinealectomy as compared with C animals. Melatonin treatment partially decreases, IRG concentration of Pn rats, and the difference between these two groups was not statistically significant. In order to obtain information about the amount of glucagon and insulin arriving at the liver from the pancreas, in ether anaesthetized rats hormone concentrations were determined in the porta vein and the inferior vena cava (Table 2). In the three groups of experimental animals there were hormone concentration gradients between the values of porta and inferior cava vein ($P < 0.01$). Although ether anaesthesia increases circulating glucagon levels in the porta and cava veins, the qualitative changes of plasma insulin and glucagon in the experimental groups were similar to those found in the jugular vein of conscious rats. Thus, a decrease of insulin and an elevation of glucagon levels in both veins of Pn rats were observed, as compared with the values obtained on C animals. In addition in Pn + Mel rats

a reduction of glucagon concentrations and an increase of insulin levels were observed.

Effect of an oral glucose overload on blood plasma glucose, insulin and glucagon levels

After an oral glucose overload in control animals (Fig. 1) glucose levels reached a peak 30 min after ingestion, thereafter decreasing to basal levels. However, in pinealectomized rats glucose intolerance was observed, with maximum glucose levels at 60 minutes after ingestion and higher values of blood glucose at 180 minutes than basal concentrations. Furthermore, melatonin treatment of Pn rats significantly improved the glucose tolerance. Regarding glucose concentrations in control animals the greater level of circulating insulin (Fig. 2, upper panel) was observed 30 minutes after the ingestion of glucose, thereafter decreasing to basal values. By contrast, in pinealectomized animals, glucose-induced insulin release was smaller ($P < 0.05$ at 15 and 30 minutes after glucose ingestion) and delayed as compared with the results obtained in the control group. However, insulin levels in the Pn + Mel rats were intermediate between the other two groups. Despite the fact that basal glucagon concentrations were significantly higher (Fig. 2, lower panel) in Pn and Pn + Mel rats in comparison to control animals ($P < 0.05$), a marked suppression of glucagon release was observed after the oral ingestion of glucose in the three experimental groups. Also, at 15, 30, 120 and 180 minutes after glucose ingestion significant differences ($P < 0.01$ and $P < 0.05$) were observed between the C animals and either Pn or Pn + Mel animals.

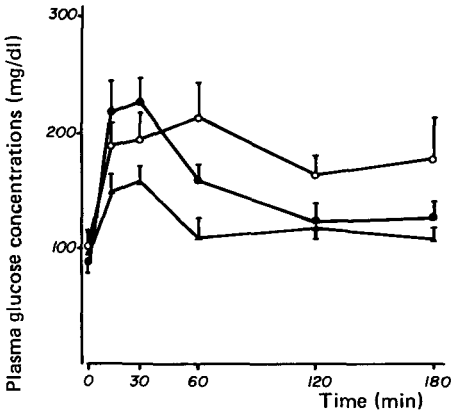

Fig. 1 Blood plasma glucose levels after oral administration of glucose to control, pinealectomized and melatonin-treated pinealectomized rats. Means ± SEM. N = 12. •—• Control; o——o pinealectomized ▲—▲ melatonin-treated pinealectomized rats.

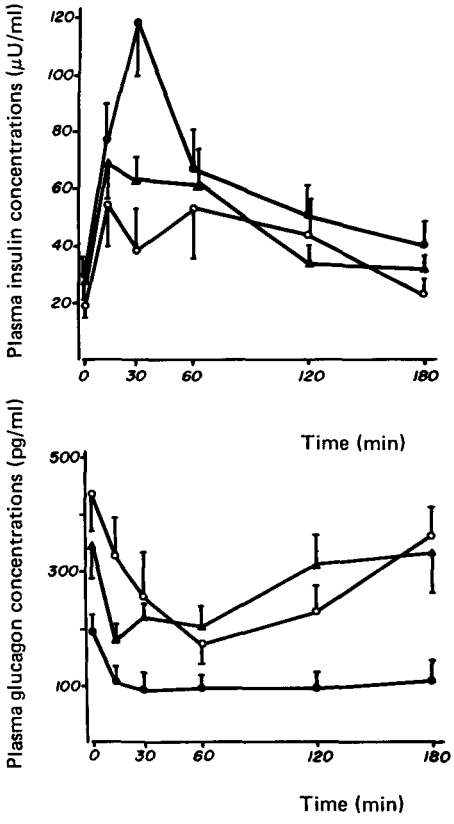

Fig. 2 Blood plasma insulin and glucagon levels after oral administration of glucose to control, pinealectomized and melatonin-treated pinealectomized rats. Means ± SEM. N = 10. •—• Control; o—o pinealectomized; ▲—▲ melatonin-treated pinealectomized rats.

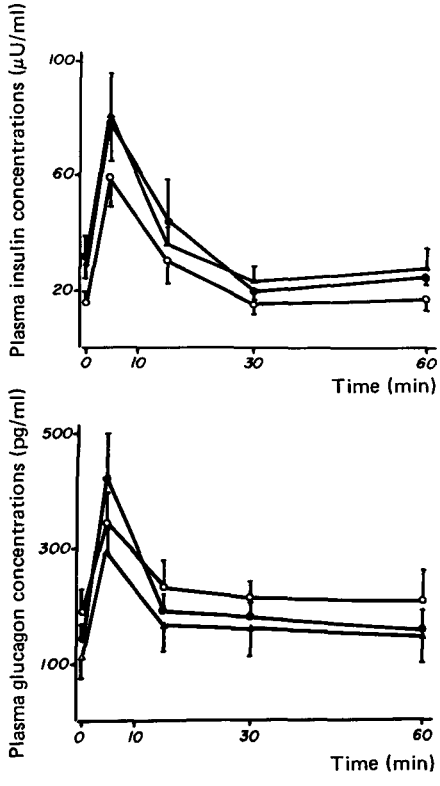

Fig. 3 Blood plasma insulin and glucagon levels after intravenous administration of arginine to control, pinealectomized and melatonin-treated pinealectomized rats. Means ± SEM. N = 14. •——• Control; o——o pinealectomized; ▲——▲ melatonin-treated pinealectomized rats.

Effect of intravenous arginine on insulin and glucagon blood plasma concentrations

As shown in Fig. 3, upper panel, the insulin response to intravenous arginine was smaller in Pn animals than in the other two groups. Again, insulin release in Pn rats was delayed, while values for control and melatonin-treated pinealectomized animals were very close.

Although glucagon basal levels were greater in Pn rats than in the other two groups (Fig. 3, lower panel) the hormone response to intravenous arginine was similar to that in the control animals. Despite the fact that glucagon levels in Pn + Mel rats after arginine administration were slightly different than in control rats, these differences were not statistically significant.

Discussion

In recent years it has been reported that a peptide extract of the bovine pineal gland has an insulin-like effect on experimental animals (*Milcu, Milcu* and *Nanu* 1963). This effect was characterized as hypoglycemia, increased glucose tolerance, glucose uptake and hepatic and muscular glycogenesis and by an improvement of the impaired biochemical manifestations of alloxan-diabetic rats. Other authors have reported (*Gorray, Quay* and *Ewart* 1979) that aliquots of the medium in which the pineal gland or sonicates of this organ had previously been incubated, have a stimulatory effect on insulin release by pancreatic islets incubated in vitro, which suggested that an active substance of the pineal gland exerts a direct effect on hormone secretion. Furthermore, a clinical entity observed in children as a recessively inherited syndrome, courses with pineal gland hyperplasia, hyperinsulinemia and insulin resistance (*Barnes, Palumbo, Hayles* and *Folgar* 1974; *West, Lloyd* and *Turner* 1975). According to these findings, in pinealectomized animals opposite effects could be anticipated; for example, glucose intolerance and reduced insulin release, which are described by us in this paper. However, our results are somewhat contradictory to the reports of other authors describing hypoglycemia and hyperinsulinemia following pinealectomy (*Csaba* and *Barath* 1971; *Milcu, Nanu-Ionesco* and *Milcu* 1971; *Nanu-Ionesco* and *Ionesco* 1969; *Nanu-Ionesco* and *Marcean* 1970; *Gorray, Quay* and *Ewart* 1979). In addition to the low basal insulin levels, we found that after stimulation with oral glucose or intravenous arginine of pancreatic B cells, insulin response was smaller, and delayed, in pinealectomized than in control rats, which could suggest either a reduction in the hormone reserve in B cells or an inhibitor effect on insulin release. Other authors have reported low insulin basal values in pinealectomized animals but described hyperinsulinemia under specified photoperiod conditions and food intake or after repeated oral glucose loads (*Milcu, Nanu-Ionesco* and *Milcu* 1971; *Gorray* and *Quay* 1977). However, the greater insulin concentrations in pinealectomized animals observed as a single determination 30 minutes after oral administration of six equal hourly doses of glucose (*Milcu, Nanu-Ionesco* and *Milcu* 1971), could represent a delayed release of insulin rather than an increase in hormone secretion. Moreover, the glucose intolerance observed by others and by us could be the final expression of a reduction of insulin release and/or the presence of circulating antagonists to the biological effects of this hormone. Thus, in pinealectomized rats there is a decrease of hepatic and muscular glycogenesis and glucose uptake (*Milcu, Milcu* and *Nanu* 1963; *Milcu, Nanu-Ionesco* and *Milcu* 1971) which is compatible with a reduced insulin release and/or biological effect and with the existence in the blood plasma of insulin antagonists that neutralize the activity of appreciable amounts of insulin (*Milcu, Nanu-Ionesco* and *Milcu* 1971) which could be responsible for a state of glucose intolerance. Also, it has been proposed that the increased release of minerals and glucocorticoids following pinealectomy could represent the agents responsible for the glucose intolerance observed in these animals (*Milcu, Nanu-Ionesco* and *Milcu* 1971). However, other antagonists to the biological action of insulin should be taken into account, such as glucagon. In fact, we found an inversion of the insulin/glucagon ratio in pinealectomized rats which could imply significant metabolic changes since both insulin and glucagon are considered as components of a bihormonal functional unit (*Unger* and *Orci* 1981) in which insulin is responsible for the storage of nutrients and where glucagon mobilizes it when the individual displays a deficient exogenous fuel availability and increased energy requirements. In addition, in several pathophysiological states (*Unger* and *Orci* 1976; *Unger* and *Orci* 1981) a reduced insulin/glucagon ratio facilitates the shift of metabolic activities to a catabolic mode and also favours the hypersecretion of contrainsular hormones, all of which is manifested as glucose intolerance, besides other metabolic changes.

To determine which pineal gland substances are able to reverse the hormonal pattern observed in pinealectomized rats, melatonin was administered daily to these animals. This replacement therapy partially restored the normal circulating levels of insulin and glucagon, suggesting that melatonin is one of the agents involved in the process, at least in the sense that due to the short half life of melatonin, replacement therapy of this hormone may not be sufficient for a complete effect to take place. In summary, our results suggest that the pineal gland is necessary to maintain normal circulating levels of insulin and glucagon and that the inversion of the insulin/glucagon ratio after pinealectomy could shift metabolic activities to a catabolic mode.

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