Studies on the Effect of Thyroxine on *in vivo* Insulin Secretion as Modified by Hypophysectomy***

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Received: May 6, 1971, accepted: September 13, 1971

Summary. Blood sugar and serum immunoreactive insulin (IRI) concentrations were measured in uninjected and thyroxine-injected hypophysectomized dogs as well as in untreated normal dogs. Thyroxine was used at the following two levels: 0.5 and 100 γ /kg body wt./day, for 10 days. Serum IRI levels in the dog in the post-absorptive conditions were unaffected by both treatments. Mildly subnormal blood sugar levels were detected in the hypophysectomized dog in the post-absorptive condition and were fully corrected by thyroxine therapy (both levels). The rate of disappearance of glucose from the blood was normal in the hypophysectomized control dogs and was unaffected by thyroxine therapy. The moderate increase in the general mean of the blood sugar throughout the test found after hypophysectomy was corrected by thyroxine (low level); thyroxine therapy (high dose) not only failed to correct the increase but in fact induced a further increase. The typical slow, low and maintained insulin response to hyperglycaemia was found in the hypophysectomized control dogs, and it remained unchanged despite the thyroxine treatment (both levels). We concluded that the presence of the pituitary gland is needed so that the thyroxine-induced inhibition of the insulin secretory response to hyperglycaemia *in vivo* may be exerted.

Etude de l'effet de la thyroxine sur la sécrétion d'insuline «in vivo», modifiée par l'ablation de l'hypophyse

Résumé. Nous avons étudié l'effet de l'hypophysectomie totale sur l'inhibition de la sécrétion de l'insuline "in vivo" par la thyroxine, au cours d'hyperglycémies provoquées, chez le chien normal, hypophysectomisé et hypophysectomisé injecté avec de la thyroxine (0.5 et 100 γ /kg/j, pendant 10 jours). On a dosé la glycémie et l'insulinémie de ces animaux, dans des conditions de base, à jeun, et après la perfusion rapide intraveineuse de glucose. On a trouvé que la glycémie de base diminue après l'hypophysectomie et qu'elle se normalise par le traitement thyroïdique. L'insulinémie de base moyenne normale, à jeun, ne subit pas de modifications à la suite des deux traitements. Pendant l'épreuve dynamique, la glycémie moyenne générale atteint un niveau plus élevé chez le chien hypophysectomisé que chez le chien normal. Tandis que la faible dose de thyroxine (0.5 γ /kg/j) peut corriger cette altération, celle-ci ne se corrige pas par la forte dose (100 γ /kg/j) qui la fait encore augmenter. Cependant, la constante k de Conard ne se modifie pas à la

suite de l'hypophysectomie chez le chien normal et elle reste normale après le traitement thyroïdique. Au cours de l'épreuve dynamique, l'insulinémie s'élève beaucoup moins, et surtout, reste plus longtemps élevée chez le chien hypophysectomisé que chez le chien témoin normal. Le traitement thyroïdique (0.5 et 100 γ /kg/j) n'a pas entraîné d'inhibition de la sécrétion de l'insuline chez le chien hypophysectomisé. En conclusion: après l'ablation totale de la glande pituitaire, la thyroxine semble avoir perdu son efficacité pour inhiber la sécrétion insulinique chez le chien.

Untersuchungen über die Thyroxinwirkung auf die „in vivo“ Sekretion von Insulin nach Hypophysectomie

Zusammenfassung. Es wurde die Wirkung der totalen Hypophysectomie auf die Hemmung der Insulinsekretion „in vivo“ untersucht. Dabei wurden an hypophysectomierten Hunden mit und ohne Thyroxin-Vorbehandlung sowie an normalen Tieren Blutzuckerwerte und Insulinkonzentrationen gemessen. Thyroxin wurde in folgenden Dosen verabreicht: 0,5 und 100 γ /kg Körpergewicht pro Tag über 10 Tage. In hypophysectomierten Hunden wurden geringfügig verminderte Blutzuckernüchternwerte gefunden, die mit einer Thyroxinbehandlung mit beiden Dosen vollständig korrigiert werden konnten. Die Seruminsulinkonzentrationen am nüchternen Hund zeigten nach beiden Behandlungen keine Veränderungen. Bei einem Belastungsversuch wurden bei hypophysectomierten Hunden höhere Blutzuckerwerte erreicht als bei normalen Hunden. Während die kleine Thyroxindosis 0,5 γ /kg diese Veränderung verhindern konnte, war die große Dosis von 100 γ /kg dazu nicht in der Lage. Sie bewirkte sogar einen weiteren Anstieg. Die Seruminsulinkonzentrationen stiegen bei den hypophysectomierten Hunden im Gegensatz zu den normalen Kontrolltieren während der Belastung weniger an, aber blieben länger erhöht. Die Thyroxinbehandlung (in der hohen und niedrigen Dosis) hat bei den hypophysectomierten Hunden keine Verminderung der Insulinsekretion bewirkt. Daraus wird die Schlußfolgerung gezogen, daß Thyroxin nach vollständiger Hypophysectomie seine dämpfende Wirkung auf die Insulinsekretion verloren zu haben scheint.

Key words: Hypophysectomized dogs-thyroxine treatment-serum immuno reactive insulin-intravenous glucose tolerance test.

* This work was partially supported by Research Grant No. 2304-B from the "Consejo Nacional de Investigaciones Científicas y Técnicas" (Argentina).

** This paper was partially presented at the Meetings of the VII Congress Intern. Diabetes Fedn, Buenos Aires, Argentina, 23—8 August, 1970.

Introduction

The thyroid hormones are the only pituitary-dependent hormones so far known to exert a moderating role on the insulin response to a glucose stimulus *in vivo*. That role was demonstrated by Andreani, Menzinger, Fallucca, Aliberti and Tamburrane (1968) in hyperthyroid patients and by Renaud, Pinto, Sverdlik and Foglia (1971a) in the experimental hyperthyroid condition in the dog. Since the actual concentration of insulin in the blood depends upon a balance between insulin secretion and its disappearance from the blood, we carried out experiments to study the second possibility as influenced by hyperthyroidism. We found that the insulin disappearance rate constant is normal in the severely hyperthyroid dog; the insulin space remained unaffected as well (Renaud, Pinto, Sverdlik and Foglia, 1971b). The first possibility would then appear to be most definitely involved, although its mechanism remains still unknown. An indirect mechanism of the thyroid hormone effect exerted *in vivo* through a sensitization of the pancreatic effectors to the inhibitory action of epinephrine on the insulin secretion was demonstrated by several investigators, and reviewed by Freychet, Rosselin, Assan and Tchobroutsky (1967). Nevertheless, recent experiments (Orsetti, Basseres and Macabies, 1970) would rather support the view of increased insulin response to hyperglycaemia during the mildly hyperthyroid condition, thereby leading to β cell exhaustion and to a poor insulin response to the glucose stimulus as severity of hyperthyroidism increases.

In a group of experiments we studied the inhibitory effect of thyroxine on insulin secretion as modified by pituitary hormones. In the present paper we report the results obtained in experiments carried out to demonstrate the effect of hypophysectomy on the changes in the action of thyroxine *in vivo* upon the insulin response to a glucose stimulus.

Material and Methods

Sixteen mongrel dogs, body weight ranging from 11.9 to 23.5 kg were used. They were fed on dog chow pellets and bones, and water *ad libitum*. Eight dogs out of the total group (dogs No. 33, 35, 41, 47, 49, 53, 55 and 57) were totally hypophysectomized (trans-temporal approach) 2–4 months prior to experiments. The remaining 8 were kept as normal control dogs (No. 13, 34, 36, 40, 42, 50b, 56 and 58).

During the experiments, the dogs were awake, fasting (18–22 h fast) and kept as calm as possible. Two blood samples were withdrawn by venipuncture; the dogs were immediately tested for intravenous glucose tolerance with simultaneous measurement of serum immunoreactive insulin (IRI). Each dog received a rapid intravenous infusion of glucose (1 g per kg body wt., in 20% (w/v) aqueous solution) and was bled 5, 15, 25, 45, 60 and 90 min after infusion. Thyrox-

ine replacement therapy was performed through chronic subcutaneous l-thyroxine¹ administration at one of the following doses: 0.5 and 100 γ /kg body wt./day, for 10 days in 2 ml saline. One hour after regimen completion a new intravenous glucose tolerance test (i.v. GTT) with simultaneous measurement of serum IRI was performed (self-control design). Serum IRI concentrations were estimated according to the Morgan and Lazarow technique, as modified by Stuart Soeldner and Slone (1965). Serum was obtained by blood clotting at room temperature (1–2 h), followed by centrifugation (5 min, 2500 r.p.m.) and storage (-15°C). The dog serum was diluted 1 : 2 and eventually 1 : 4 for insulin assay; ox insulin² was employed as a standard. The following system was used for the assay: 1) undiluted rabbit anti (Guinea pig total serum); 2) Guinea pig anti (ox insulin) serum, 1 : 2000 dilution; 3) tracer: ¹²⁵I-insulin. Reagents No. 1 and 2 were prepared in our laboratories; radioactive insulin was purchased at the Radiochemical Centre, Amersham, Bucks., England. The results of serum IRI measurements were expressed as microunits per milliliter serum. A previously fluorinated sample of the same blood was used for sugar determination (Nelson, 1944); the results were expressed as milligrams reducing substances per 100 ml of blood.

The results of either fasting blood sugar or serum IRI determinations (fasting or during the i.v. GTT) were statistically analyzed for variance, randomized-block design (Lison, 1958); general mean of the blood sugar after glucose injection and Conard's *k* constant per group (Conard, 1955) were calculated according to Bancroft (1960) and further analyzed (Student *t*-test).

Results

The results of measurements of fasting blood sugar are shown in table 1. The typical significant decrease in the levels of fasting blood sugar was found in hypophysectomized dogs prior to thyroxine therapy. The therapy (0.5 and 100 γ /kg body wt.) enhanced the blood sugar values ($P < 0.05$ and $P < 0.01$ compared with uninjected hypophysectomized dogs), the normal levels being achieved. The fasting serum IRI concentration was found to be unaffected by hypophysectomy either followed by thyroxine replacement therapy (both doses) or not.

Fig. 1 shows the results of serum IRI and blood sugar assay during the i.v. GTT in normal, hypophysectomized and thyroxine-injected (0.5 γ /kg body wt.) hypophysectomized dogs. The constants for glucose disappearance from blood in the same animals

¹ Synthetic l-thyroxine (sodium salt) provided by Glaxo Laboratories (Argentina); it was dissolved with the help of some drops of N NaOH and further diluted with saline.

² Crystalline glucagon-free insulin, potency 27.5 I.U./mg protein, provided by Hoechst Laboratories (Argentina), through the kindness of Dr. H. Schneider.

(*k*) are shown in table 1. A mildly overnormal blood sugar curve was found after hypophysectomy ($P < 0.01$); however, glucose disappeared from blood at a normal rate. Thyroxine therapy corrected the increased blood sugar levels throughout the test with no change in *k* glucose thereby suggesting the possibility of a rising effect on the glucose space rather than on glucose utilization.

response to hyperglycaemia was detected just 15 min after glucose infusion ($57 \mu\text{U/ml}$, $P < 0.05$); the maximum was achieved just 25 min after glucose load ($63 \mu\text{U/ml}$). In thyroxine-injected hypophysectomized dogs, the increase in serum IRI levels was found 25 min after infusion ($P < 0.05$), rapidly returning to base line. The thyroxine therapy failed to induce any change in the low and slow insulin response to intra-

Table 1. Fasting serum IRI and blood sugar levels and Conard's *K* constant in the hypophysectomized dog as modified by thyroxine therapy. The figures in the table mean average $\pm$ S.E.; *N*: number of cases; columns No. 1 and 2 belong to post-absorptive condition

Dog. No.	Condition	Dose of thyroxine γ/kg body wt./day for 10 days	N	Serum IRI $\mu\text{U/ml}$		Blood sugar $\text{mg}/100 \text{ ml}$		<i>k</i>
34-36-40-42-50b	Normal	0	5	10 $\pm$ 4	28 $\pm$ 11	70 $\pm$ 5	71 $\pm$ 4	0.0138
33-35-41-47-49	Hypox.	0	5	15 $\pm$ 8	14 $\pm$ 9	63 $\pm$ 3 ^a	59 $\pm$ 2 ^a	0.0114
33-35-41-47-49	Hypox.	0.05	5	28 $\pm$ 10	18 $\pm$ 12	76 $\pm$ 6	71 $\pm$ 4	0.0188
13-56-58	Normal	0	3	24 $\pm$ 7	17 $\pm$ 9	76 $\pm$ 3	72 $\pm$ 9	0.0156
53-55-57	Hypox.	0	3	25 $\pm$ 14	17 $\pm$ 3	53 $\pm$ 3 ^a	46 $\pm$ 9 ^a	0.0145
53-55-57	Hypox.	100	3	24 $\pm$ 10	9 $\pm$ 8	61 $\pm$ 5	68 $\pm$ 3	0.0094

^a $P < 0.02$ compared with normal control dogs; all the other comparisons are not significant.

Fig. 1. Effect of the thyroxine replacement therapy on serum IRI and blood sugar levels in the hypophysectomized dog during the i.v. GTT. Thyroxine dose: 0.5 γ/kg body wt./day, for 10 days. In parenthesis, number of animals per group

The insulin response to hyperglycaemia was typical in both normal and hypophysectomized control groups (see the bottom half of figure 1). In normal dogs, a five-fold increase in serum IRI levels above post-absorptive values was detected 5 min after glucose infusion ($105 \mu\text{U/ml}$, $P < 0.001$). They were still higher than base line ($P < 0.01$) 10 minutes later. In uninjected hypophysectomized dogs, the insulin

venously-induced hyperglycaemia in the hypophysectomized dog, whatever point of the serum IRI curve after glucose load is being considered ($P > 0.05$).

The results of serum IRI and blood sugar assays during the i.v. GTT in normal, hypophysectomized dogs and in thyroxine-injected (100 γ/kg body wt.), hypophysectomized dogs are shown in figure 2. The constants for glucose disappearance from the blood in the same animals (*k*) are shown in table 1. The average blood sugar after glucose i.v. injection was hardly overnormal in this group of hypophysectomized dogs.

Fig. 2. Effect of the thyroxine replacement therapy on serum IRI and blood sugar levels in the hypophysectomized dog during the i.v. GTT. Thyroxine dose: 100 γ/kg body wt./day, for 10 days. In parenthesis, number of animals per group

Thyroxine, at this level, was not only unable to correct it, but rather induced a further increase ($P < 0.01$ compared with the normal control dogs). It would appear then that thyroxine therapy at a high level reduces the glucose space in the hypophysectomized dog. *k* glucose was normal in untreated hypophysectomized dogs and was unaffected by thyroxine therapy.

As shown in the bottom half of figure 2, the typical serum IRI curve during an i. v. GTT was found in the normal control group: 5 minutes after glucose infusion, serum IRI rose four-fold (to 81 μ U/ml) over post-absorptive values ($P < 0.01$); 25 minutes after glucose load, the serum IRI was still above post-absorptive levels, only this difference was not significant. In the uninjected hypophysectomized group, the insulin response to hyperglycaemia was low and slow; it achieved its maximum just 45 minutes after glucose infusion (42 μ U/ml) and immediately returned to post-absorptive levels. The thyroxine therapy (high dose) also failed to induce any significant change in the insulin response to the glucose stimulus, whatever point of the serum IRI curve after glucose load is being considered ($P > 0.05$).

Discussion

The results reported here demonstrate that the presence of the pituitary gland is needed so that thyroxine may induce a decrease in the insulin response to a glucose stimulus. Our results confirm previous reports about the low anti-insulinic effect of the thyroid hormones themselves. The combined treatment of thyroid feeding and injection of anterior pituitary extract induce the development of a diabetic condition in the normal dog, which is not observed after chronic administration of just thyroid gland (Houssay, 1945a). Furthermore: a previous treatment with anterior pituitary extracts sensitizes the pancreas to the diabetogenic action of the thyroid feeding in the partially depancreatized dog (Houssay, 1945b).

In our experiments, the rate of disappearance of glucose from the blood after glucose load was unaffected by thyroxine therapy in the hypophysectomized dog. Since the serum IRI levels also remained unchanged after therapy, we may then conclude that the typical higher-than-normal insulin sensitivity to endogenous insulin in the hypophysectomized dog failed to be changed by chronic thyroxine therapy. This is in close agreement with the results of experiments carried out by several investigators (Soskin, Levine and Heller, 1939; De Bodo and Sinkoff, 1953; De Bodo and Altszuler, 1958) who reported that the thyroid hormone treatment is unable to correct the hypersensitivity to exogenous insulin in the hypophysectomized dog.

Soskin, Levine and Heller (1939) found that thyroxine therapy corrects the tendency to hypoglycaemic

crisis in the hypophysectomized dog during starvation, through a relatively greater increase in the rate of formation than in the rate of utilization of blood sugar. The enhancement of the fasting (18–22 h fast) blood sugar levels in the hypophysectomized dog following thyroxine therapy that we are reporting here confirms their findings.

Our results reported in the present paper, led us to conclude that the thyroxine-inhibitory action on the insulin response *in vivo* to hyperglycaemia, disappears after hypophysectomy in the dog, thus suggesting a significant interaction between thyroxine and hypophyseal hormones on the regulation of insulin secretion.

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