Effects of Nembutal and ether anaesthesia on gluconeogenesis *in vivo* in the rat

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Anaesthesia is known to affect different aspects of intermediary metabolism (Brunner *et al.*, 1975), and Nembutal and diethyl ether have been specifically shown to enhance plasma glucose (Arola *et al.*, 1981; Furner *et al.*, 1972) and to decrease plasma gluconeogenic amino acid concentrations, accompanied by minor changes in liver glycogen concentration (Arola *et al.*, 1981). Although these modifications produced by Nembutal and ether suggest a possible enhancement of gluconeogenesis, this has not been clearly established, and it has been proposed that anaesthetics stimulate glycogenolysis and glycolysis and inhibit gluconeogenesis (Brunner *et al.*, 1975). The present investigation was performed to study the effect of Nembutal anaesthesia on gluconeogenesis *in vivo* in the rat and to determine whether this effect was dependent on dietary status.

Fed and 24h-starved female Wistar rats of 180–200g body wt. were intraperitoneally injected with either sodium Nembutal (33mg/kg body wt.) or saline (0.9% NaCl). After 30min, the animals received a pulse of [U-^{14}C]alanine [10μCi (0.2mmol)/200g body wt.] through a tail vein. Blood samples were collected from the tip of the tail 2 and 5min later, the animals were decapitated at 10min, and a piece of liver was immediately frozen in liquid N_2. Blood [^{14}C]glucose and liver [^{14}C]glycogen and [^{14}C]lipids were purified and assayed by slight modifications of previously described methods (Herrera *et al.*, 1969).

The conversion of administered [U-^{14}C]alanine into circulating [^{14}C]glucose and ^{14}C-labelled liver glycogen and lipids did not differ in fed animals between the Nembutal-treated subjects and their saline-treated controls (Table 1). After 24h of starvation there was increased conversion of [^{14}C]alanine into circulating glucose and liver lipids (corresponding in more than 94% to triacylglycerol glycerol in the starved animals) in both Nembutal-treated and control rats (Table 1), with significantly higher values in the first group. In the Nembutal-treated starved rats there was also increased radioactivity in liver [^{14}C]glycogen (Table 1) and, although liver glycogen concentration increased in this group as compared with controls, both circulating glucose and liver glycogen specific radioactivities were significantly greater in the Nembutal-treated animals.

To determine whether this gluconeogenic effect of Nembutal in the starved animals was dependent on the substrate, the same protocol was followed, but with [3-^{14}C]pyruvate instead of [^{14}C]alanine as tracer. Nembutal also produced an enhancement in the formation of circulating [^{14}C]glucose and liver [^{14}C]glycogen and [^{14}C]lipids from [3-^{14}C]pyruvate in the 24h-starved rats (Table 1). Ether anaesthesia instead of Nembutal in the 24h-starved rat also enhanced the formation of circulating [^{14}C]glucose (Table 1) and increased blood [^{14}C]-glucose specific radioactivity from [3-^{14}C]pyruvate, but did not affect the formation of either liver [^{14}C]glycogen or [^{14}C]lipids (Table 1) when values were compared with their respective controls. The enhancing effect of either Nembutal or ether on the production of [^{14}C]glucose in the fasted rat cannot be attributed to the action of these anaesthetics, decreasing the utilization of the newly formed glucose for two reasons: (i) anaesthetics have been reported to enhance, not to decrease, glycolysis (Brunner *et al.*, 1975), which in any case would augment the disappearance of the newly formed glucose, causing underestimation of [^{14}C]glucose production in anaesthetized rats; and (ii) differences between anaesthetized rats and their controls were observed very shortly (2min) after administration of the traces, before values could be masked by any potential difference in utilization of the [^{14}C]glucose formed.

Present results indicate that Nembutal does not alter gluconeogenesis in the fed animal, and that in the starved animal both Nembutal and ether have a stimulatory effect, which may contribute to the hyperglycaemia produced by these anaesthetics. The different response to Nembutal in fed and starved animals has also been reported for the insulinotropic effects of glucose administration in the rat (Aynsley-Green *et al.*, 1973). Both gluconeogenesis and pancreatic insulin release are known to be influenced by catecholamines, and it has been shown that the sympathoadrenal activity is affected both by Nembutal anaesthesia (Traynor & Hall, 1981) and by starvation (Young & Landsberg, 1979). Thus differences in the metabolic response to Nembutal anaesthesia produced by dietary status may be secondary to its positive action of increasing the sympathetic efferent activity.

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Table 1. *Effects of anaesthetics on the appearance of [^{14}C]glucose in the circulation and on hepatic glycogen and lipids after the intravenous administration of either [U-^{14}C]alanine or [3-^{14}C]pyruvate in the fed or 24h-starved rat*

Radioactivity values have been adjusted to an initial value of 1×10^6d.p.m./200g body wt. for the injected tracer. Glucose space (ml) used for calculations was 38% of body wt. *P* values are for statistical comparisons versus controls: N.S., not significant ($P > 0.05$).

	Circulating [^{14}C]glucose (d.p.m./200g body wt.)			Liver [^{14}C]glycogen (d.p.m./g)	Liver [^{14}C]lipids (d.p.m./g)
Time after tracer (min) ...	2	5	10	10	10
[U-^{14}C]Alanine					
Fed, controls	179 ± 8	193 ± 13	362 ± 38	88 ± 3	371 ± 68
Fed, Nembutal	195 ± 14	236 ± 14	303 ± 27	71 ± 26	255 ± 84
P	N.S.	N.S.	N.S.	N.S.	N.S.
24h-starved, control	274 ± 18	414 ± 67	1036 ± 171	81 ± 3	513 ± 97
24h-starved, Nembutal	445 ± 27	1015 ± 61	2258 ± 128	239 ± 46	1024 ± 108
P	<0.001	<0.001	<0.001	<0.01	<0.01
[3-^{14}C]Pyruvate					
24h-starved, controls	361 ± 22	662 ± 53	1724 ± 72	130 ± 14	752 ± 55
24h-starved, Nembutal	1250 ± 75	1905 ± 293	2997 ± 43	632 ± 174	1093 ± 137
P	<0.001	<0.01	<0.001	<0.001	<0.05
24h-starved, ether	841 ± 148	1178 ± 114	2987 ± 147	140 ± 22	1011 ± 185
P	<0.01	<0.01	<0.001	N.S.	N.S.

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Biological potencies of different insulins in isolated adipocytes

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Knowledge of the molecular basis of insulin action remains incomplete (Czech, 1981), and changes in insulin sensitivity are believed to take place at the level of the target tissue (Olefsky, 1981). For adipose tissue, the functional heterogeneity of insulin receptors has been emphasized (Olefsky & Chang, 1979), but, on the basis of lipogenic and anti-lipolytic effects of chemically modified insulins, it has been proposed that different effects of the hormone in adipocytes are mediated through the same set of receptors (Thomas *et al.*, 1979). To study this problem of adipose-tissue insulin sensitivity further, we determined the dose–response relationships of different types of insulins on CO_2 production and fatty acid synthesis from [U-^{14}C]glucose in basal conditions and triacylglycerol glycerol formation from [1-^{14}C]glycerol in the presence of adrenaline by isolated adipocytes. This latter parameter was selected because we previously had found (Dominguez & Herrera, 1976) that adrenaline decreased [^{14}C]triacylglycerol glycerol synthesis from [1-^{14}C]-glycerol by adipose tissue *in vitro*, an effect significantly compensated for by insulin when glucose was absent from the incubation media. Thus insulin effects on glucose utilization and on glycerol conversion into triacylglycerol glycerol in the presence of adrenaline may be considered independent parameters.

Adipocytes were isolated with collagenase in the presence of ovomucoid trypsin inhibitor by a modification (Bellido & Herrera, 1978) of the method of Rodbell (1964) from epididymal fat-pads of fed male Wistar rats. Isolated adipocytes were placed in vials containing 1 ml of Krebs–Ringer bicarbonate buffer, pH 7.4, supplemented with purified bovine serum albumin (10 mg/ml) and either [U-^{14}C]glucose (1 μCi/ml, 5 mM) or [1-^{14}C]glycerol (1 μCi/ml; sp. radioactivity 31 Ci/mol) plus adrenaline bitartrate (1.4 μM). Bovine, pig and rat insulin (from Novo Industri A/S, Bagsvaerd, Denmark) and bonito insulin (from Kodama Ltd., Tokyo, Japan) were radioimmunoassayed against rat insulin standards and added to the corresponding incubation vial at concentrations of 0, 0.001, 0.01, 0.1, 1, 10, 100, 200 or 1000 μunits/ml. Incubations were performed for 120 min under O_2/CO_2 (19 : 1), and samples were processed as previously described (Bellido & Herrera, 1978; Dominquez & Herrera, 1976).

Insulin potency corresponding to the concentration of the hormone required to produce a half-maximal effect was estimated from the curves of log dose against the percentage response. Curves were linearized by using log–logit transformation by plotting log insulin dose on the abscissa versus $\ln[(R_i - 100)/(R_m - R_i)]$ on the ordinate scale, where R_i denotes response as a percentage of basal (100%, no insulin in the medium) and R_m denotes the maximal insulin response. Linear correlation coefficients against 0 were estimated by Student's t test, and comparisons of sensitivity values for the different insulins and parameters studied were performed by the analysis of variance for two factors and the Tukey t test (Sokal & Rohlf, 1969).

Insulin biological potencies found in the present study are summarized in Table 1. Compiled mean values of the effects of the four types of insulin used on formation of either CO_2 and fatty acid from [^{14}C]glucose or triacylglycerol glycerol from [^{14}C]glycerol in the presence of adrenaline were very similar and did not differ statistically, indicating that the sensitivity of all four insulins was the same for each of the three parameters studied. There were, however, differences among the insulins, the bonito type being the least active, because a greater concentration of it was required than of the other types to obtain a half-maximal effect, this difference being statistically significant (Table 1). There were, however, no differences in the observed biological potencies of rat, pig and bovine insulin.

Present results support the hypothesis that once insulin is recognized by (and presumably bound to) its appropriate receptor, it produces an unique metabolic response in the adipocyte.

Table 1. *Biological potencies of different insulins in isolated adipocytes*

Values are the concentrations of the hormone (μunits/ml) required to produce half-maximal effect. Correlation coefficients of the log–logit transformations against 0 were always $P < 0.001$ (no. of pairs = 39–62/group). Analysis of the variance among the three parameters was not significant, but $F = 8.77$ (d.f. 3;6, $P < 0.05$) among values for bovine, pig, and rat insulins versus bonito insulin.

Substrate ...	[U-^{14}C]glucose		[1-^{14}C]glycerol	
Insulin	$^{14}CO_2$	^{14}C-labelled fatty acids	^{14}C-labelled triacylglycerol glycerol	Means ± S.E.M.
Bovine	8.02	6.36	4.52	6.3 ± 1.0
Pig	4.10	6.54	3.40	4.7 ± 1.0
Rat	2.47	3.96	4.31	3.6 ± 0.6
Bonito	15.92	13.60	26.96	18.8 ± 4.1

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