Effects of Anesthetics and Starvation on In Vivo Gluconeogenesis in Virgin and Pregnant Rats

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To study in vivo gluconeogenesis, female virgin rats were injected intravenously with ^{14}C-alanine (ul) and the production of ^{14}C-glucose was determined at two, five, or ten minutes thereafter. At ten minutes the appearance of ^{14}C-glycogen in the liver was also determined. The intraperitoneal injection of sodium pentobarbital (Nembutal) (33 mg/kg body weight) 30 minutes prior the tracer did not affect the rate of gluconeogenesis in fed rats compared with unanesthetized animals, whereas in rats fasted 24 hours it produced a significant enhancement in all parameters studied. A similar effect in enhancing in vivo gluconeogenesis was observed with both pentobarbital or ether anesthesia when 3-^{14}C-pyruvate was used as tracer in virgin rats fasted 24 hours. In contrast to the effect in virgin animals, pentobarbital anesthesia did not modify in vivo gluconeogenesis in either fed or 24-hour fasted 21-day pregnant rats. Ether anesthesia, however, caused an enhancement in ^{14}C-glucose production from 3-^{14}C-pyruvate in 24-hour fasted pregnant rats. On the basis of reported changes in sympathoadrenal activity produced by starvation and pregnancy, present results indicate that the enhancing effects of anesthetics on gluconeogenesis result from their capacity to stimulate adrenal medulla cathecholamine release or tissue sympathetic activity. Our findings also demonstrate that in an investigation of metabolic parameters it cannot be assumed that effects of anesthetics are always of the same degree and direction since they vary with the condition of the experimental subject.

UNDER certain conditions both sodium pentobarbital (Nembutal) and diethyl ether anesthesia are known to produce hyperglycemia,[1-4] to impair glucose tolerance,[5-8] to stimulate hepatic glycogenolysis,[9,10] and to affect the concentration of glycolytic intermediates in liver.[11-13] It has been also proposed that these anesthetics inhibit gluconeogensis,[14] but this effect has not been tested directly. Although some of these metabolic effects of anesthetics have been demonstrated in vitro, some of their in vivo effects may be secondary to their primary actions on sympathoadrenal activity.[15-17] This possibility is substantiated by the fact that the response of in vivo glucose disposition to ether or pentobarbital anesthesia differs for fed and fasted animals,[6] and it is known that fasting reduces the sympathetic nervous activity in the rat.[18] We have previously shown that urinary catecholamine excretion in the fasting rat at late gestation is markedly different from that observed in nonpregnant animals,[19] and others have been proposed that this difference corresponds to a dissociation in adrenal medullary and sympathetic nervous system responses to hypoglycemia in the fasting pregnant rat.[20] Since the rate of in vivo gluconeogenesis is unchanged in the fed pregnant rats but greatly enhanced in fasted rats compared with virgin controls,[21] effects of anesthetics on gluconeogenesis could differ not only with food intake but also with gestation. The subject deserves attention as both of these anesthetics are widely used for metabolic studies in the rat. In the present study of the effects of pentobarbital and ether anesthesia on in vivo gluconeogenesis, results were substantially different in fed and 24-hour fasted 21-day pregnant and virgin rats.

MATERIALS AND METHODS

Sprague-Dawley female rats were mated when they reached 160 g. and gestation was timed from the appearance of spermatozoids in vaginal smears. Sex- and age-matched virgin rats were studied in parallel. Animals were housed in collective cages in a temperature-controlled room (23 ± 1 °C) with a 12 hour on-off light cycle and fed ad libitum with Purina chow pellets (Purina). Fed or 24-hour fasted pregnant rats at day 21 of gestation and their respective virgin controls were injected intraperitoneally with either sodium pentobarbital (Nembutal) (33 mg/kg body weight) or saline (0.9% NaCl) and 30 minutes thereafter they received through the tail vein a pulse injection of either ^{14}C-alanine (ul) or 3-^{14}C-pyruvate (10 µCi. 0.2 mmol/200 g body weight) (Radiochemical Center, Amersham, Bucks, UK). Other animals were placed in a large glass beaker containing cotton swabs soaked with diethyl ether that were protected with a plastic grid on the bottom and covered with glass plate. After five minutes, at which time the animals were anesthetized, they were intravenously injected with 3-^{14}C-pyruvate. Blood samples were collected from the tip of the tail two and five minutes after the tracer was given. Animals were decapitated at ten minutes and a piece of liver was immediately placed into liquid nitrogen while blood was collected from the wound into heparinized containers. Blood aliquots were deproteinized with Ba(OH)$_2$-ZnSO$_4$[22] for glucose determination[23] and for passing over anionic-cationic microcolumns to purify ^{14}C-glucose, using the slightly modified procedure described previously.[23] Recovery of ^{14}C-glucose added to blood before precipitation was more than 98.7% by this technique, whereas the recoveries of added ^{14}C-alanine (ul) and 3-^{14}C-pyruvate were less than 0.19% and 0.22%, respectively. Aliquots of the frozen liver were precipitated from alkali digests,[24] and after being reprecipitated twice with ethanol they were hydrolyzed with 2.5 mol L H$_2$SO$_4$ for two hours at 100 °C for counting and analyzed for glucose with glucose oxidase, as described previously.[23] Values for ^{14}C-glucose formation were calculated by considering a "glucose space" of 38.7

of body weight, and all radioactive values were adjusted to an initial value of 1×10^6 dpm/200 g body weight for the injected tracer Statistical comparison among the groups was done by Student's t test.

RESULTS

Production of ^{14}C-glucose at short times after the intravenous administration of ^{14}C-alanine (ul) was studied in fed female virgin unanesthetized rats and in others under pentobarbital anesthesia. As shown in Table 1, the appearance of ^{14}C-glucose in the blood increased progressively in both groups from two to ten minutes after tracer administration. Pentobarbital anesthesia did not affect this parameter, and it had no effect on either blood glucose concentration or specific activity at any of the times studied, on the appearance of radioactivity in liver glycogen, or on liver glycogen concentration (Table 1). When the same experiment was performed on unanesthetized rats fasted for 24 hours, ^{14}C-glucose production was significantly enhanced (v fed rats) at two, five, and ten minutes after the tracer administration (Table 1). There was also a significant reduction in blood glucose concentration, an increase in blood ^{14}C-glucose specific activity, a decrease in liver glycogen concentration, and an enhancement in liver ^{14}C-glycogen specific activity in the fasted unanesthetized v fed rats (Table 1). In contrast to the lack of effect in fed animals, in the 24-hour fasted rats pentobarbital anesthesia significantly enhanced the rate of gluconeogenesis from

^{14}C-alanine (ul), as shown by increases in ^{14}C-glucose production and ^{14}C-glucose specific activity in blood at two, five, and ten minutes after administration of tracer (Table 1). Pentobarbital anesthesia also augmented liver glycogen concentrations and ^{14}C-glycogen levels and decreased ^{14}C-glycogen specific activity in the liver of the 24-hour fasted animals at ten minutes after the tracer (Table 1). To determine whether the effects of pentobarbital on gluconeogenesis in the 24-hour fasted rats were dependent on the nature of the substrate used, the same experiment was performed with 3-^{14}C-pyruvate as tracer, and results are shown in Table 2. Effects of pentobarbital anesthesia on in vivo gluconeogenesis from 3-^{14}C-pyruvate were even greater than those observed with ^{14}C-alanine (ul), as shown by the great increase in the formation of ^{14}C-glucose, the significant increase in blood glucose concentration at two and five minutes after the administration of the substrate, and the increase in blood ^{14}C-glucose specific activity (Table 2). Pentobarbital anesthesia also greatly enhanced the appearance of radioactivity in liver glycogen and the liver glycogen concentration, and it decreased liver ^{14}C-glycogen specific activity at ten minutes after 3-^{14}C-pyruvate administration in the 24-hour fasted rats (Table 2). To determine whether ether anesthesia also affected the rate of gluconeogenesis in the 24-hour fasted animals, parallel experiments using 3-^{14}C-pyruvate as tracer were performed in animals anesthetized with ether. As shown in Table 2, ether anesthesia enhanced in vivo

Table 1. Effects of Pentobarbital Anesthesia on the Formation of ^{14}C-Glucose and Hepatic ^{14}C-Glycogen From ^{14}C-Alanine (ul) in Fed and 24-Hour Fasted Virgin Rats*

		Blood			Liver		
	Minutes After Giving Tracer	Formation of ^{14}C-Glucose (dpm × 10^{-2}/200 g body wt)	Glucose Concentration (mg/dL)	^{14}C-Glucose Specific Activity (dpm/mg)	^{14}C-Glycogen (dpm/g)	Glycogen Concentration (%)	^{14}C-Glycogen Specific Activity (dpm/mg)
Fed rats							
Controls	2	179 ± 8	97 ± 11	289 ± 7			
	5	193 ± 13	83 ± 6	316 ± 21			
	10	362 ± 38	113 ± 8	427 ± 51	88 ± 3	3.22 ± 0.15	2.7 ± 0.3
Pentobarbital	2	195 ± 14	82 ± 1	304 ± 24			
	5	236 ± 14	82 ± 1	475 ± 91			
	10	303 ± 27	95 ± 3	413 ± 34	72 ± 26	3.10 ± 0.20	2.6 ± 1.2
Rats fasted for 24 hr							
Controls	2	274 ± 18††	63 ± 7††	605 ± 53††			
	5	414 ± 67†	66 ± 5†	952 ± 129††			
	10	1036 ± 171**	86 ± 4††	1613 ± 244**	81 ± 3	0.004 ± 0.001††	2237 ± 620*
Pentobarbital	2	445 ± 27***	66 ± 2†††	894 ± 72**			
	5	1015 ± 61***	69 ± 2†††	1891 ± 51***			
	10	2258 ± 128†††**	80 ± 2†††	3529 ± 139††***	239 ± 46†**	0.130 ± 0.020†††***	149 ± 27†††*

*Rats were injected intraperitoneally with either pentobarbital (33 mg/kg body weight) or saline (controls) and 30 minutes thereafter received a pulse of ^{14}C-alanine (µl) (10 µCi, 0.2 mmol/200 g body weight) through a tail vein. Animals were bled from the tip of the tail at two and five minutes after the tracer and were sacrificed at ten minutes for purification and analysis of ^{14}C-glucose in blood and ^{14}C-glycogen in liver, as indicated in Material and Methods section. Values correspond to mean ± SEM of five to ten rats/group. Statistical comparisons between pentobarbital-anesthetized and control rats are denoted by asterisks, while those between fasted and fed animals are indicated by crosses: * or † = $P < 0.05$; ** or †† = $P < 0.01$; *** or ††† = $P < 0.001$.

Table 2. Effects of Pentobarbital and Ether Anesthesia on the Formation of ^{14}C-Glucose and Hepatic ^{14}C-Glycogen From 3-^{14}C-Pyruvate in 24-Hour Fasted Virgin Rats*

	Minutes After Giving Tracer	Blood			Liver		
		Formation of ^{14}C-Glucose (dpm × 10^{-2}/200 g body wt)	Glucose Concentration (mg/dL)	Glucose Specific Activity (dpm/mg)	^{14}C-Glycogen (dpm/g)	Glycogen Concentration (%)	^{14}C-Glycogen Specific Activity (dpm/mg)
Controls	2	361 ± 22	65 ± 5	706 ± 53			-
	5	662 ± 53	63 ± 4	1247 ± 34			
	10	1724 ± 72	93 ± 6	2347 ± 192	130 ± 14	0.010 ± 0.003	1097 ± 253
Pentobarbital	2	1250 ± 75***	94 ± 1**	1746 ± 283**			
	5	1905 ± 293**	101 ± 1***	2863 ± 259***			
	10	2997 ± 43***	113 ± 3	3429 ± 121**	632 ± 174***	0.230 ± 0.0208***	240 ± 66*
Ether	2	841 ± 148**	84 ± 4*	1346 ± 287			
	5	1178 ± 114**	80 ± 3**	1858 ± 208*			
	10	2987 ± 147***	98 ± 2	3621 ± 350**	140 ± 22	0.010 ± 0.001	1664 ± 175

*Rats were injected intraperitoneally with either pentobarbital (33 mg/kg body weight) or saline (controls) and 30 minutes thereafter were injected intravenously with pulse of 3-^{14}C-pyruvate (10 μCi, 0.2 mmol/200 g body weight) through a tail vein. Other animals were placed in an ether atmosphere and five minutes therafter were injected with the tracer. Animals were bled from the tip of the tail at two and five minutes after receiving tracer and were sacrificed at ten minutes for purification and analysis of ^{14}C-glucose in blood and ^{14}C-glycogen in liver, as indicated in Material and Methods section. Values correspond to mean ± SEM of five to ten rats/group. Statistical comparisons between pentobarbital- or ether-anesthetized and control rats are denoted by asterisks: * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$.

gluconeogenesis in the 24-hour fasted rats, although their effects were less pronounced than those of pento-barbital, as shown by the smaller increases in ^{14}C-glucose production, in blood glucose concentration, and in blood ^{14}C-glucose specific activity, with no significant effects on either liver ^{14}C-glycogen, gly-cogen concentration, or ^{14}C-glycogen specific activity.

Similar series of experiments were conducted in fed and 24-hour fasted 21-day pregnant rats. As shown in Table 3, pentobarbital anesthesia did not affect any of

the gluconeogenetic parameters studied in fed preg-nant rats using ^{14}C-alanine (ul) as substrate, and results were similar to those in fed virgin rats (Table 1). In comparison with unanesthetized fed rats, 24-hour fasted pregnant rats (Table 3) showed a signifi-cant increase in the rate of ^{14}C-glucose production, a reduction in blood glucose concentration, an increase in ^{14}C-glucose specific activity, and no change in the appearance of ^{14}C-glycogen in liver, with an intense reduction in the concentration of liver glycogen and an

Table 3. Effects of Pentobarbital Anesthesia on the Formation of ^{14}C-Glucose and Hepatic ^{14}C-Glycogen From ^{14}C-Alanine (ul) in Fed and 24-Hour Fasted 21-Day Pregnant Rats*

	Minutes After Giving Tracer	Blood			Liver		
		Formation of ^{14}C-Glucose (dpm × 10^{-2}/200 g body wt)	Glucose Concentration (mg/dL)	^{14}C-Glucose Specific Activity (dpm/mg)	^{14}C-Glycogen (dpm/g)	Glycogen Concentration (%)	^{14}C-Glycogen Specific Activity (dpm/mg)
Fed rats							
Controls	2	184 ± 4	57 ± 4	435 ± 35			
	5	187 ± 18	60 ± 4	437 ± 46			
	10	229 ± 24	69 ± 8	447 ± 70	95 ± 23	2.59 ± 0.18	3.0 ± 0.6
Pentobarbital	2	206 ± 10	61 ± 2	415 ± 17			
	5	209 ± 9	60 ± 3	451 ± 31			
	10	258 ± 33	62 ± 4	498 ± 95	66 ± 16	3.00 ± 0.10	2.3 ± 0.6
Fasted for 24 hr							
Controls	2	282 ± 30*	44 ± 4*	937 ± 117††			
	5	483 ± 58††	47 ± 4*	1253 ± 149††			
	10	1203 ± 166†††	56 ± 2	2844 ± 404†††	98 ± 14	0.016 ± 0.005†††	1627 ± 927
Pentobarbital	2	267 ± 18††	40 ± 2††	977 ± 105††			
	5	399 ± 20†††	45 ± 2††	1204 ± 143†††			
	10	1118 ± 181†††	55 ± 2	2664 ± 409†††	304 ± 8.6*	0.070 ± 0.020*	333 ± 57†††

*Rats were injected intraperitoneally with either pentobarbital (33 mg/kg) or saline (controls) and 30 minutes thereafter received a pulse of ^{14}C-alanine (ul) (10 μC, 0.2 mmol/200 g body weight) through a tail vein. Animals were bled from the tip of the tail at two and five minutes after receiving the tracer and were sacrificed at ten minutes for purification and analysis of ^{14}C-glucose in blood and ^{14}C-glycogen in liver, as indicated in Material and Methods section. Values correspond to mean ± SEM of five to ten rats/group. Statistical comparisons between pentobarbital-anesthetized and control rats are denoted by asterisks, whereas those between fasted and fed animals are indicated by crosses: * or † = $P < 0.05$; ** or †† = $P < 0.01$; *** or ††† = $P < 0.001$.

increase in liver ^{14}C-glycogen specific activity. These changes produced by fasting in pregnant unanesthetized rats (Table 3) were of the same magnitude as those found in virgin animals (Table 1), with the exception of lower concentration of blood glucose in the pregnant animals, in which there was greater circulating ^{14}C-glucose specific activity. In contrast to the fasted virgin animals, in the fasted 21-day pregnant rats pentobarbital anesthesia did not affect the parameters studied, with the sole exception of a slight but significant increase in the appearance of ^{14}C-glycogen and glycogen concentration in the liver (Table 3). When using 3-^{14}C-pyruvate instead of ^{14}C-alanine (ul) as substrate for studying in vivo gluconeogenesis in the 24-hour fasted rats, it was seen that the rate of ^{14}C-glucose formation was greater in 21-day pregnant rats (Table 4) than in virgin rats (Table 1), in agreement with previous findings.[21] As with ^{14}C-alanine (ul), pentobarbital anesthesia did not affect either the formation of ^{14}C-glucose, the blood glucose concentration, or ^{14}C-glucose specific activity from 3-^{14}C-pyruvate in the 24-hour fasted pregnant rats (Table 4). Pentobarbital anesthesia significantly enhanced the appearance of radioactivity in liver glycogen and the liver glycogen concentration, decreasing liver ^{14}C-glycogen specific activity in the 24-hour fasted pregnant rats ten minutes after the administration of 3-^{13}C-pyruvate (Table 4). When 24-hour fasted 21-day pregnant rats were studied under ether anesthesia, the production of ^{14}C-glucose, blood glucose concentration, and blood ^{14}C-glucose specific activity were significantly higher than in unanesthetized rats (Table 4), without liver glycogen concentration or ^{14}C-glycogen liver content being affected (Table 4).

These results parallel the effects in 24-hour fasted virgin animals under ether anesthesia (Table 2).

Owing to the different responses to anesthesia in fed or fasted pregnant and virgin rats, the comparison between these groups differed substantially according to the conditions used. In Fig. 1, values of ^{14}C-glucose formation at two minutes after the gluconeogenetic substrate was injected are compared for pregnant and virgin rats. With ^{14}C-alanine (ul) as substrate (Fig. 1A), values did not differ between unanesthetized pregnant and virgin rats when fed or fasted for 24 hours, the increase in ^{14}C-glucose production being similar in both fasted pregnant and virgin rats. When the animals were studied under pentobarbital anesthesia, ^{14}C-glucose production from ^{14}C-alanine (ul) at two minutes did not differ between fed pregnant and virgin rats but it was significantly lower in the pregnant v the virgin rats when studied after 24 hours of fasting (Fig. 1A), owing to the lack of pentobarbital effect in the pregnant rats and the rise in ^{14}C-glucose production in virgins. Using 3-^{14}C-pyruvate as substrate (Fig. 1B), ^{14}C-glucose production at two minutes was significantly higher in unanesthetized pregnant v virgin rats. Pentobarbital anesthesia reversed that difference by increasing ^{14}C-glucose production in virgins without affecting pregnant subjects, making the difference between both groups statistically significant but in the opposite direction than when the study was performed in unanesthetized rats (Fig. 1B). Ether anesthesia produced a parallel stimulatory effect of ^{14}C-glucose production in both groups of 24-hour fasted rats compared with their unanesthetized controls, and values in pregnant rats were higher, although not significantly so, than in virgins (Fig. 1B).

Table 4. Effects of Pentobarbital and Ether Anesthesia on the Formation of ^{14}C-Glucose and Hepatic ^{14}C-Glycogen From 3-^{14}C-Pyruvate in 24-Hour Fasted 21-day Pregnant Rats Rats*

| | Minutes After Giving Tracer | Formation of ^{14}C-Glucose (dpm × 10^{-2}/200 g body wt) | Blood | | Liver | | |
			Glucose Concentration (mg/dL)	^{14}C-Glucose Specific Activity (dpm/mg)	^{14}C-Glycogen (dpm/g)	Glycogen Concentration (%)	^{14}C-Glycogen Specific Activity (dpm/mg)
Controls	2	546 ± 82	45 ± 2	1383 ± 192			
	5	1023 ± 111	50 ± 2	2445 ± 158			
	10	1979 ± 81	57 ± 3	4539 ± 192	166 ± 19	0.020 ± 0.007	1235 ± 213
Pentobarbital	2	708 ± 51	54 ± 5	1603 ± 94			
	5	1186 ± 53	58 ± 6	2789 ± 363			
	10	2113 ± 72	71 ± 6	4247 ± 500	771 ± 212**	0.180 ± 0.040***	489 ± 153*
Ether	2	1222 ± 198**	59 ± 3**	2704 ± 502*			
	5	2094 ± 149***	68 ± 4***	4229 ± 486**			
	10	2965 ± 138***	79 ± 5**	5211 ± 374*	191 ± 21	0.010 ± 0.003	1452 ± 341

*Rats were injected intraperitoneally with either pentobarbital (33 mg/kg) or saline (controls) and 30 minutes thereafter were injected intravenously with a pulse of 3-^{14}C-pyruvate (10 μCi/0.2 mmol/200 g body weight) through a tail vein. Other animals were placed in an ether atmosphere and five minutes thereafter were injected with the tracer. Animals were bled from the tip of the tail at two and five minutes after receiving the tracer and were sacrificed at ten minutes for purification and analysis of ^{14}C-glucose in blood and ^{14}C-glycogen in liver, as indicated in Material and Methods section. Values correspond to mean ± SEM of five to ten rats/group. Statistical comparisons between pentobarbital- and ether-anesthetized and control rats are denoted by asterisks: * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$.
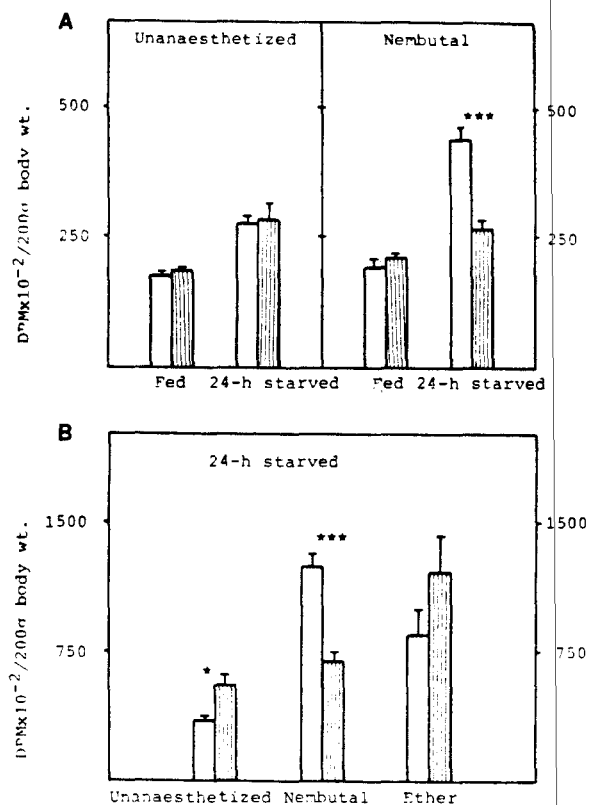
Fig. 1. Comparison of ^{14}C-glucose formation between 21-day pregnant (hatched bars) and virgin rats (open bars) two minutes after the intravenous administration of either ^{14}C-alanine (ul) (A) or 3-^{14}C-pyruvate (B). Fed or 24-hour fasted rats were anesthetized by the intraperitoneal injection of sodium pentobarbital (Nembutal) (33 mg/kg body weight) or by placing them in an ether atmosphere. Unanesthetized animals received an intraperitoneal injection of saline. At 30 minutes after the administration of pentobarbital or saline, or five minutes after being placed in ether atmosphere, animals were injected intravenously with 10 μCi/0.2 mmol/200 g body weight of either ^{14}C-alanine (ul) (Fig. 1A) or 3-^{14}C-pyruvate (Fig. 1B). Results correspond to the two-minute values of the formation of ^{14}C-glucose (see Tables 1 to 4). Statistical comparisons between pregnant and virgin animals are denoted by asterisks: * = $P < 0.05$; *** = $P < 0.001$.

DISCUSSION

Present results show that while pentobarbital anesthesia does not affect in vivo glucose formation from alanine in fed virgin rats, pentobarbital and ether anesthesia enhance gluconeogenesis from both alanine and pyruvate in 24-hour fasted rats. These anesthetics are known to impair glucose tolerance in the fasted rat[6] and could modify glucose turnover. Since the observed effects on ^{14}C-glucose formation were already present two minutes after administration of the substrate, we concluded that they were not an indirect consequence of actions reducing peripheral utilization of the newly formed glucose but resulted from enhanced gluconeogenesis. These effects must be responsible for the hyperglycemia found in the fasted anesthetized rats, which had very low liver glycogen stores that were not

further depleted by the anesthetics. These results are in agreement with the increase in circulating glucose levels found in rats after administration of these anesthetics,[1,3,4,6] although the amount of change differed according to the doses used and the dietary conditions of the animals. A greater response to ether and pentobarbital anesthesia in the fasted v the fed rats has been reported for the impairment of intravenous glucose tolerance,[6] suggesting that their effects on carbohydrate metabolism are secondary to neuroendocrine responses, which differ according to dietary conditions. It has been proposed that the hyperglycemic effects of both ether and pentobarbital are secondary to those enhancing catecholamine release[25] or related to the sympathetic innervation of the liver.[8] In agreement with this hypothesis, urinary excretion of norepinephrine and tissue sympathetic activity have been found to be suppressed in fasted v fed rats,[18,19] indicating that the anesthetics overcome this suppression and allow maximal gluconeogenesis in the fasted rat. Further support for this hypothesis is provided by present results showing that, contrary to results in virgin animals, pentobarbital anesthesia does not affect in vivo gluconeogenesis in fasted pregnant rats. Urinary excretion of catecholamines is greatly augmented in the fasted pregnant rats v virgin controls[19] as a consequence of enhanced adrenal medulla activity,[20] causing a depletion in their catecholamine content.[20] In this way, the lack of effect of pentobarbital anesthesia on in vivo gluconeogenesis in the fasted pregnant rat could be explained by the incapacity of pentobarbital to enhance the release of catecholamine from the adrenals, whose pool has been depleted as a consequence of the intense hypoglycemia present in the fasted mother. This explanation is not valid for the effects of ether anesthesia, as it was found that, unlike the case with pentobarbital, ether enhanced the rate of in vivo gluconeogenesis from pyruvate in fasted pregnant and virgin rats. Owing to the known dissociation between adrenal medullary and sympathetic nervous system responses to fasting,[20] ether anesthesia in the starved animal differs from pentobarbital in that it may modify gluconeogenesis by affecting the tissue sympathetic activity, which is diminished by fasting in both pregnant and nonpregnant rats.[20]

The action of anesthetics on hormones such as glucocorticoids, known potentially to affect gluconeogenesis, may be partly responsible for the different reactions to anesthesia evoked in pregnant and nonpregnant rats. Plasma glucocorticoid levels increase both in pregnancy[21,22] and under anesthesia,[23,24] but the delayed action of steroid hormones on glucose metabolism probably precludes their involvement during the brief period of anesthesia used in the present study.

Although the precise mechanism for these differences in the gluconeogenetic response to pentobarbital and ether anesthesia remains to be determined, it is evident from the present study that their effects vary greatly with the condition of the animal. This means that in investigating parameters of carbohydrate metabolism (and presumably metabolism of other substrates) in anesthetized animals it cannot be assumed that the effects of anesthesia are of the same degree and direction in the different experimental conditions used; these effects must be tested each time for every new circumstance and parameter.

ACKNOWLEDGMENTS

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