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Effect of Anesthetic or Analgesic Drugs on Lipogenic and Lipolytic Adipose Tissue Activities[1] (41573)

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Abstract. The effects of several anesthetic or analgesic agents (halothane, Na thiopental, ketamine, nitrous oxide, xylazine, and procaine) administered *in vivo*, on swine adipose tissue metabolism were measured. Adipose tissue samples were collected from the subcutaneous depot before and after administration of the anesthetic or analgesic agent. The rate of oxidation of glucose to CO_2 and the lipogenic rate, or the basal and stimulated lipolytic rates, were measured on tissue slices incubated *in vitro*. There were no effects on the oxidation of glucose to CO_2 or the incorporation into lipids except by procaine. Procaine depressed ($P \leq 0.05$) the lipogenic rate (12%) and tended ($P \leq 0.1$) to depress the oxidative rate (9%). The only observable effect on the lipolytic rates was a tendency ($P \leq 0.1$) for ketamine to increase the stimulated lipolytic rate. The minimal effects on adipose tissue metabolism reported herein were obtained with short times of compound administration. Several agents (nitrous oxide, procaine, and xylazine) are not recommended because of poor analgesia.

The predominant animal model for the study of adipose tissue metabolism has been the rat; tissue samples have been obtained generally after killing. The use of larger animals, such as swine, as model species precludes sacrifice to obtain a small tissue sample because of animal cost. Small biopsy samples (about 1 g) of subcutaneous adipose tissue have been obtained from swine in several laboratories. However, the need for larger tissue samples and the increased concern for animal welfare issues require the use of anesthetic or analgesic agents before the biopsy procedure. Since anesthetic or analgesic agents are not pharmacologically benign, the effect on the metabolic pathways of interest in a particular tissue must be known before use of a drug. I have tested the effect of several anesthetic or analgesic agents administered *in vivo* on lipid metabolism in swine subcutaneous adipose tissue obtained by biopsy techniques.

Materials and Methods. Crossbred pigs (Yorkshire × Landrace or Chester White × Large White) were raised under normal husbandry conditions in temperature-controlled buildings. They were fed *ad libitum* a corn–soybean meal-based diet (ca. 18% protein) from weaning at 4 weeks of age. On a given experimental day, two female and two castrated male pigs, each from a different litter, were used to test a single anesthetic or analgesic agent. An adipose tissue biopsy sample was obtained (1) from each animal in the dorsal neck region with a spring-loaded biopsy gun without anesthetic or analgesic, the agent was administered and then a second biopsy sample was obtained. The control animals were biopsied twice with an interval of 3 min.; no anesthetic or analgesic agent was administered. The tissue samples were placed in room temperature 0.9% NaCl, tissue slices (0.3 mm) were prepared and either glucose oxidative plus lipogenic rates or lipolytic rates were measured on a given experimental day. The wounds were treated once with wound powder (Tylan plus Neomycin Eye Powder, Elanco Product, Eli Lilly Co., Indianapolis, Ind.). Halothane (Halocarbon Laboratories Inc., Hackensack, N.J.) was administered with a face mask and initially vaporized at 3% with maintenance at 0.5% in the presence of 100% O_2 flowing at 1.5 liter/min. Sodium thiopental (Pentothal, Abbott Laboratories, North Chicago, Ill.) was administered intravenously into an ear vein as a 100 mg/ml solution at

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TABLE I. EFFECT OF ANESTHETIC OR ANALGESIC AGENTS ON ADIPOSE TISSUE LIPOGENESIS

			Glucose metabolism rate[c]					
			Oxidation to CO_2			Incorporation into lipid		
Agent	Interval[a]	Weight[b]	Initial rate	Change	P[d]	Initial rate	Change	P[d]
Control	180	19.1 ± 0.2	8.4 ± 0.9	0.1 ± 0.4	0.72	10.5 ± 1.5	−0.1 ± 0.5	0.80
Halothane	190	16.4 ± 1.4	8.8 ± 2.7	1.3 ± 1.0	0.28	21.4 ± 6.3	3.3 ± 2.3	0.26
Na thiopental	70	17.2 ± 1.2	9.2 ± 1.0	−1.0 ± 0.7	0.28[e]	21.7 ± 2.4	1.9 ± 5.3	0.74
Ketamine	140	16.8 ± 1.2	8.8 ± 1.1	0.1 ± 0.9	0.98	21.9 ± 2.9	0.4 ± 3.9	0.92
Nitrous oxide	180	15.9 ± 0.2	8.5 ± 0.4	−0.4 ± 0.2	0.12	15.2 ± 2.7	−0.3 ± 0.2	0.22
Xylazine	900	20.8 ± 1.5	6.0 ± 0.6	0.1 ± 0.1	0.40	7.6 ± 0.8	−0.3 ± 0.5	0.62
Procaine	170	18.6 ± 0.4	10.7 ± 0.8	−1.0 ± 0.4	0.10	13.7 ± 1.2	−1.7 ± 0.5	0.04

[a] Interval (in seconds) between administration of agent and completion of the biopsy following agent administration.
[b] kg, mean ± SE.
[c] Micromoles glucose incorporated into CO_2 or total lipids per 2 hr per gram tissue; mean ± SE. The rates from the initial biopsy sample and the change observed between it and the postanesthetic or -analgesic sample are indicated.
[d] Probabilities are for the two-tailed test of the hypothesis of equality of paired pre- and postdrug samples.
[e] $n = 3$ rather than usual 4.

11 mg/kg body wt. Ketamine HCl (Ketaset, Bristol Laboratories, Syracuse, N.Y.) was administered intravenously into an ear vein as a 100 mg/ml solution at 5 mg/kg body wt. Xylazine (Rompun, Haver-Lockhart, Shawnee, Kans.) was administered intramuscularly as a 100 mg/ml solution at 6 mg/kg body wt. Procaine HCl (Frank Veterinary Laboratories, Inc., Edina, Minn.) was infused subcutaneously at the periphery of an area approximately 5 to 6 cm in diameter as a 25 mg/ml solution; the terminal biopsy was taken from the center of this area. Nitrous oxide was administered with a face mask at 80% in the presence of 100% O_2 flowing at 1.5 liter/min.

The maximal rates of [U-^{14}C]glucose incorporation into lipids and CO_2 were determined in a medium of Krebs–Ringer bicarbonate buffer, 20 mM glucose (0.5 μCi ^{14}C), and 100 mU insulin/ml. The glucose concentration was chosen to saturate the metabolic pathways and achieve maximal rates (2). Insulin has essentially no effect on these aspects of glucose metabolism in swine adipose tissue *in vitro*, but is routinely added to high concentrations to ensure measurement of maximal metabolic rates (2). One hundred milligrams of slices (0.3 mm thick) were incubated in quadruplicate in a total volume of 3 ml at 37°C for 120 min in a shaking water bath (90 strokes/min) in sealed flasks under an atmosphere of 95% O_2–5% CO_2. The reactions were stopped with H_2SO_4, the CO_2 was trapped in a suspended center well, and the lipid was extracted with chloroform and methanol from the tissue plus the medium. The radioactivity in CO_2 and lipid was determined. Detailed methods were described previously (2).

Lipolysis was measured by incubation of 100 mg adipose tissue slices at 37°C with shaking (90 strokes/min) for 90 min. The medium (3 ml total volume) was Krebs–Ringer bicarbonate containing 4% fatty acid-poor Fraction V bovine albumin (Sigma Chemical Co., St. Louis, Mo.), 5.56 mM glucose, and 0.57 mM ascorbate. The nonstimulated or basal lipolytic rate was measured in triplicate in the above medium whereas the maximal stimulated rate was measured in triplicate in the presence of 10^{-4} M epinephrine bitartrate plus 10^{-3} M theophylline. The high epinephrine concentration plus theophylline were present to ensure measurement of the maximal lipolytic rate (3). The lipolytic rate was assessed by extraction and titrimetric determination of the fatty acids released to the medium. Detailed methods were described previously (3).

Results. The anesthetic or analgesic effectiveness of the pharmacological agents tested was variable. Halothane and Na thiopental yielded recumbancy of the pig as did ketamine although there was considerable spontaneous muscle movement with ketamine. Xylazine produced only moderate analgesia. Nitrous oxide yielded recumbancy but some apparent pain perception. Procaine produced

TABLE II. EFFECT OF ANESTHETIC OR ANALGESIC AGENTS ON ADIPOSE TISSUE LIPOLYSIS

			Lipolytic rate[c]					
			Basal			Net stimulated		
Agent	Interval[a]	Weight[b]	Initial rate	Change	P^d	Initial rate	Change	P^d
Control	180	25.8 ± 0.9	8.3 ± 0.5	−0.5 ± 1.2	0.70	54.5 ± 6.1	−9.0 ± 5.3	0.18
Halothane	120	20.7 ± 0.4	10.1 ± 0.6	−0.2 ± 2.0	0.92	70.0 ± 6.7	−8.9 ± 12.0	0.52
Na thiopental	30	21.4 ± 0.6	12.9 ± 0.9	−0.2 ± 3.4	0.94	57.1 ± 3.4	9.2 ± 6.3	0.28[e]
Ketamine	130	24.1 ± 1.0	3.6 ± 0.5	3.4 ± 3.7	0.42	33.7 ± 3.6	9.9 ± 3.6	0.08
Nitrous oxide	165	27.3 ± 0.2	14.7 ± 1.3	1.2 ± 2.3	0.64	49.7 ± 2.9	0.4 ± 7.7	0.96
Xylazine	900	26.4 ± 0.8	5.4 ± 1.7	1.1 ± 1.2	0.42	51.7 ± 1.4	3.0 ± 3.5	0.48[e]
Procaine	165	26.9 ± 0.9	4.3 ± 1.9	−1.2 ± 2.3	0.64	56.9 ± 7.5	0.6 ± 6.0	0.92

[a] Interval (in seconds) between administration of agent and completion of the biopsy following agent administration.
[b] kg, mean ± SE.
[c] Micromoles fatty acid liberated per 2 hr per gram tissue, mean ± SE: basal = rate without exogenous hormones; net stimulated = rate in presence of 10^{-4} *M* epinephrine plus 10^{-3} *M* theophylline minus basal rate. The rates from the initial biopsy sample and the change between it and the postanesthetic or -analgesic sample are indicated.
[d] Probabilities are for the two-tailed test of the hypothesis of equality of paired pre- and postdrug samples.
[e] $n = 3$ rather than usual 4.

only moderate analgesia (infused peripherally to the biopsy in an attempt to limit the concentration in the biopsy sample). The time to achieve recumbancy for all agents except xylazine (im injection) was relatively short (Tables I and II).

The only effect of any analgesic or anesthetic agent on glucose metabolism (Table I) was the depression (12%) by procaine of glucose incorporation into lipids ($P \leqslant 0.05$) and the tendency toward depression (9%) of glucose oxidation ($P \leqslant 0.1$). No agent affected the basal lipolytic rate (Table II). Likewise, the stimulated rate of lipolysis was not affected but tended to be increased (29%) by ketamine, ($P \leqslant 0.1$).

The use of anesthetic or analgesic agents allowed the removal of large samples of adipose tissue; several approaches were attempted with Na thiopental anesthesia in pigs at about 20 kg body weight. The tissue was not used for the metabolic experiments reported herein. Six biopsy samples were removed in 3 min from the time of anesthetic adminstration with one person doing all biopsy procedures (2 min if one person operated the biopsy gun and another removed the tissue samples). The pigs were given 3 ml of antibiotic, intramuscularly (Pen-mycin, RXV Veterinary Products, Porterville, Calif.) for these and other procedures producing extensive wounds. Healing of these multiple wounds (about 0.5 to 1.0 cm apart) was rapid with or without treatment with wound powder. Large, contiguous pieces of adipose tissue (about 2 × 6 cm) were removed from the mid-dorsal line in the neck region by surgical dissection from the skin and underlying muscle. The use of a single incision was troublesome for tissue dissection but healing was rapid. Retention of the skin intact on one 6-cm side allowed easy dissection and good healing if sutures were close (four to five per 6-cm side and two per short side). Retention of intact skin on one short side allowed easy dissection, but 50% or more of the skin became necrotic; healing was otherwise satisfactory. The skin could also be totally removed with satisfactory healing.

Discussion. Numerous types of compounds, alone or in combination, have been used to produce anesthesia or analgesia in pigs (4–7). The agents used in this study were chosen for diversity of drug type coupled with ease of administration. The general inhalation anesthetics, nitrous oxide and halothane, the intravenous dissociative anesthetic, ketamine, the hypnotic–sedative barbiturate, thiopental, and the intramuscular sedative–analgesic, xylazine were tested. In addition, the subcutaneous infused local anesthetic, procaine was studied. Only single drugs were used in this study so that the observations were not confounded by drug combinations.

Some anesthetic or analgesic agents, present in *in vitro* incubations, influenced the lipolytic rate of adipose tissue. Local anesthet-

ics (e.g., procaine, dibucaine, lidocaine) inhibited lipolysis (8–10). Halothane stimulated lipolysis of adipose tissue at low *in vitro* concentration (11, 12) whereas the lipolytic activity was suppressed at very high halothane concentrations (12). Induction of anesthesia with hexobarbital, halothane, and nitrous oxide did not affect basal or stimulated fatty acid release from subsequently biopsied adipose tissue (10). However, halothane (16) and ether (17) induced anesthesia both stimulated *in vivo* release of fatty acids. I am not aware of studies on the influence of anesthetics on lipogenic rates. However, the numerous metabolic effects reported for many anesthetic and analgesic agents preclude indiscriminate use of any drug without testing to determine the influence on the enzymes or pathways being studied. For example, ketamine uncoupled mitochondrial oxidative phosphorylation (13), xylazine, *in vivo*, caused hyperglycemia and hypoinsulinemia in beef cattle (14), and pentobarbital, compared to ether *in vivo*, depressed rat liver glycogen; whereas the reverse was observed in guinea pigs (15).

In contrast to the rather profound metabolic effects reported for various anesthetic or analgesic agents, the short term *in vivo* presentation of similar agents in the current study produced minimal effects on swine adipose tissue metabolism. Since the time between administration of the drug and the subsequent biopsy was short for all compounds except xylazine, more profound effects might be expected with a longer duration between drug administration and biopsy. Longer exposure to the drug would presumbly produce greater adipose tissue concentrations although this would depend on the pharmacokinetic and tissue distribution properties of each drug in swine. The results reported for swine adipose tissue may or may not be extrapolated to other tissues or species.

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