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The Effects of Halothane and Succinylcholine on Oxygen Uptake of the Canine Gracilis Muscle

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The effects of halothane and succinylcholine (SCh) on $\dot{V}_{O_2}$ and blood flow of the canine gracilis muscle were determined and compared with previous findings for the gastrocnemius muscle. Because halothane decreased and SCh increased the $\dot{V}_{O_2}$'s of the two muscles to approximately the same extent, the responses of the two muscles were averaged and used to estimate the contribution of change in skeletal muscle $\dot{V}_{O_2}$ to change in whole-body $\dot{V}_{O_2}$. These projections suggested that increasing halothane from 0.1 to 0.8 per cent would reduce skeletal muscle $\dot{V}_{O_2}$ approximately 12 per cent and comprise a decrease of 4 per cent in whole-body $\dot{V}_{O_2}$. Infusion of SCh, 0.3 mg/kg/min, was estimated to increase skeletal muscle $\dot{V}_{O_2}$ approximately 30 and 14 per cent during the first and second hours, respectively, and to contribute increases in whole-body $\dot{V}_{O_2}$ of 11 and 5 per cent, respectively. The accuracy of the latter estimates was supported by previous direct determinations of the effects of SCh on whole-body $\dot{V}_{O_2}$. Whereas the gracilis and gastrocnemius muscles were similar in these responses, actual resting $\dot{V}_{O_2}$ and blood flow were significantly less for the gracilis muscle, as were cytochrome c concentration, succinic dehydrogenase activity, and $\dot{V}_{O_2}$ *in vitro*. (Key words: Skeletal muscle O_2 consumption; Halothane; Succinylcholine; Cytochrome c; Succinic dehydrogenase.)

THE RATE of O_2 consumption ($\dot{V}_{O_2}$) of the canine gastrocnemius muscle is decreased by halothane and increased by succinylcholine.[1, 2] These findings cannot safely be extrapolated to skeletal muscle generally because of the many differences among individual muscles of the same species, including variations in structure, composition, innervation, responses to nervous stimuli, and resting $\dot{V}_{O_2}$.[3] Nor can the overall effect on skeletal muscle be directly determined, because total skeletal muscle $\dot{V}_{O_2}$ cannot be measured at the present time. The issue is regarded as important, however, because skeletal muscle $\dot{V}_{O_2}$ is the greatest single component of whole-body $\dot{V}_{O_2}$ and has been variously estimated to comprise 30 to 40 per cent of total $\dot{V}_{O_2}$.[4, 5]

The present studies offer an indirect approach to the question of the nature of the general responses of skeletal muscle; we have determined the effects of halothane and succinylcholine (SCh) on another canine muscle, the gracilis, which has a resting $\dot{V}_{O_2}$ considerably less than that of the gastrocnemius muscle (approximately 0.3 and 1.0 ml/min/100 g, respectively).[1, 2, 6, 7] Because in the present studies gracilis muscle $\dot{V}_{O_2}$ values decreased with halothane and increased with SCh in approximately the same proportions as gastrocnemius muscle $\dot{V}_{O_2}$ values, it seems likely that the combined findings can be extrapolated, percentage-wise, to skeletal muscle generally. In addition, the relative difference between the $\dot{V}_{O_2}$'s of the gracilis and gastrocnemius muscles was confirmed, and supporting evidence for the validity of these observed differences was obtained in the form of differences between cytochrome c concentrations, succinic dehydrogenase activities, and $\dot{V}_{O_2}$'s *in vitro*.

Materials and Methods

For the determination of gracilis muscle $\dot{V}_{O_2}$, five unpremedicated dogs weighing 15 to 22 kg were anesthetized with halothane and the tracheas intubated with the aid of 1 to 4 mg of SCh. Pulmonary ventilation with 35 per cent O_2 in N_2 was provided by a Harvard

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TABLE 1. Values in Canine Gracilis and Gastrocnemius Muscles at Rest at 37 C and during Halothane Anesthesia (1.7 ± 0.1 Per Cent Expired)

	Gracilis*		Gastrocnemius†	
	Mean	SE	Mean	SE
$\dot{V}_{O_2}$ (ml/min/100 g)	0.31‡	0.03	1.05	0.06
Flow (ml/min/100 g)	5.6‡	0.6	31.2	2.7
P_{O_2} (muscle venous) (mm Hg)	41‡	1	55	2
Excess lactate produced (per cent)§	15	1	14	1
Arterial pressure (mean) (mm Hg)	91	4	93	2
Pa_{O_2} (mm Hg)	160	11	165	5
Pa_{CO_2} (mm Hg)	35	2	37	1
Hb (g/100 ml)	12	1	13	1
Weight (wet) (g)	39‡	3	64	3

* Present study, ten muscles in five dogs.
† Previous studies.[1,2]
‡ Significantly different from gastrocnemius muscle value ($P < 0.05$) by Student's t test, unpaired data.
§ Expressed in O_2 equivalence units relative to total muscle $\dot{V}_{O_2}$.

pump, adjusted to result in a Pa_{CO_2} of 30 to 45 mm Hg (electrodes, 37 C). The average mean expired halothane concentration, by infrared analyzer, was 1.7 ± 0.1 per cent. There were no spontaneous movements. Catheters were placed in the carotid artery and in one or more peripheral veins for sampling, pressure measurements, and infusion of fluids and drugs. Core temperature (right atrial) was maintained at 37.0 ± 0.1 C by electric blankets and heat lamps. The venous drainages of the right and left gracilis muscles were isolated and, after heparinization and cannulation, collected separately, with provision for muscle venous blood sampling and determination of flow rate by timed collections. Muscle temperature was maintained at 37.0 ± 0.1 C by heat lamps.

These preparations were followed by a one-hour period during which control values were determined. After this, SCh was infused intravenously in amounts previously demonstrated to result in total paralysis (0.3 mg/kg/min) and an increase in gastrocnemius muscle $\dot{V}_{O_2}$.[2] The response to SCh was followed for two hours, after which halothane concentration was reduced to 0.1 ± 0.1 per cent, expired, and maintained at this level for an additional hour while maintaining the infusion of SCh.

Gracilis muscle $\dot{V}_{O_2}$ was calculated by the Fick formula from venous flow rate and A–$\dot{V}_{O_2}$ and expressed relative to the wet weight of the muscle removed at autopsy. Blood O_2 content was calculated from P_{O_2} (electrodes at 37 C) and hemoglobin and oxyhemoglobin (IL CO-Oximeter, Model 182), as validated by comparison with Van Slyke-Neill determinations.[8] Arterial pressure was transduced by strain gauge. Arterial and muscle venous blood lactate and pyruvate concentrations were determined by enzymatic methods. Excess lactate production by muscle and the O_2 equivalence of excess lactate produced were calculated as suggested by Miller.[9] Values reported for $\dot{V}_{O_2}$ are based on determinations of flow and A–$\dot{V}_{O_2}$ each five minutes. Other measurements were less frequent but all spanned the period of observation.

In additional studies, the right and left gastrocnemius and gracilis muscles of eight dogs anesthetized with halothane (1.7 per cent, expired) were removed and prepared for histochemical studies and determination of cytochrome c content (C-c), succinic dehydrogenase activity, and $\dot{V}_{O_2}$ *in vitro*. Samples were from the midportion of the belly of the muscle and from adjacent tissue toward the insertion. Each was analyzed in duplicate. There were only small, insignificant differences among the findings at the three sites, and all data have been averaged to yield a single value for each muscle. The staining technique of Nachlas and associates[10] for the demonstration of succinic dehydrogenase was used in the histochemical studies.

TABLE 2. Cytochrome c Content, Succinic Dehydrogenase Activity, and $\dot{V}_{O_2}$ *in vitro* of Canine Gracilis and Gastrocnemius Muscles (16 Each, Eight Dogs)

	Gracilis		Gastrocnemius	
	Mean	SE	Mean	SE
Cytochrome c (μg/g)	149*	7	179	8
Succinic dehydrogenase activity (mg formazan/g)	2.8*	0.2	3.9	0.3
$\dot{V}_{O_2}$ (μl/g/min)	8.8*	0.6	13.6	1.0

* Significantly different ($P < 0.05$), Student's *t* test, paired data.

A modification of the method of Margoliash and Walasek[11] was used for the determination of C-c, using standards prepared daily by solution of C-c (*Candida krusei*, Cal Biochem) in dilute NaOH of *p*H 9.0, with analysis in duplicate by means of a recording spectrophotometer, using the difference between absorbances at 550 and 560 mμ. In 24 studies utilizing samples of muscle with and without added standard, the yields averaged 85 ± 16 per cent. Final values were corrected to reflect the retention of solvent by pellets but not the average yield. Succinic dehydrogenase activity was determined with a modification of the method of Kun and Abood,[12] using standards prepared in the range of 5 to 50 μg of INT formazan per milliliter of ethyl acetate and readings at 490 mμ in a spectrophotometer (Gilford 300N). $\dot{V}_{O_2}$ *in vitro* was determined at 37 C with the aid of a Model KM Oxygraph equipped with a Clark-type electrode (Gilson Medical Electronics, Middleton, Wis.). After a two-minute temperature equilibration of the muscle homogenate (0.9 ml), 10 μmoles of sodium succinate and 1 μmole of adenosine diphosphate were added, and $\dot{V}_{O_2}$ was followed for at least 10 minutes.

Results

In the present study, mean gracilis muscle $\dot{V}_{O_2}$ was 0.31 ± 0.03 ml/min/100 g, compared with 1.05 ± 0.06 ml/min/100 g previously determined for the gastrocnemius muscle during conditions that were similar in all known respects, including halothane concentration (1.7 per cent), absence of SCh, temperature, arterial pressure, Pa_{O_2}, Pa_{CO_2}, and Hb (table 1). The significantly lower $\dot{V}_{O_2}$ of

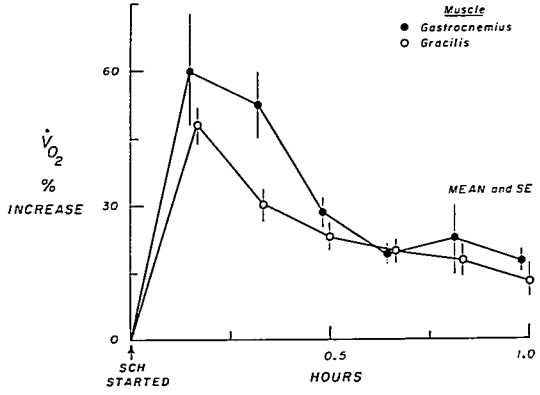

FIG. 1. Responses of gracilis and gastrocnemius muscle $\dot{V}_{O_2}$'s to infusion of succinylcholine (SCh). Note the similar patterns and magnitudes of increase in $\dot{V}_{O_2}$ for the two muscles.

gracilis muscle was associated with a lesser blood flow rate, absolutely as well as relative to muscle $\dot{V}_{O_2}$. Although venous P_{O_2} was lower for the gracilis muscle, this was without apparent effect on the patterns of metabolic pathways utilized, because lactate production occurred in each gracilis muscle and at a relative rate, having an average value not significantly different from that previously determined for the gastrocnemius muscle. Accordingly, these muscles differ not only in aerobic but also in total (aerobic plus anaerobic) metabolic activity. The wet weight of the whole gracilis muscle was less than that of the gastrocnemius muscle for dogs of similar total body weight (15 to 22 kg, present study; 14 to 23 kg, previous study).

These differences between $\dot{V}_{O_2}$ values in gracilis and gastrocnemius muscles *in vivo* during halothane anesthesia were paralleled by differences in composition and aerobic activity *in vitro* (table 2). In these studies, gracilis as compared with gastrocnemius muscle had a lesser C-c concentration, less succinic dehydrogenase activity, and a lower $\dot{V}_{O_2}$ *in vitro*. Succinic dehydrogenase activity was also less apparent in the histochemical studies of the gracilis muscle, with a predominance of A fibers and fewer of the B and C fibers of Stein and Padykula,[13] as compared with the gastrocnemius muscle.

With SCh and unchanged halothane (1.7 per cent, expired), gracilis muscle $\dot{V}_{O_2}$ and $A\text{–}\dot{V}_{O_2}$ increased in each muscle studied. The pattern of an early peak increase in $\dot{V}_{O_2}$, followed by a lesser, sustained increase, was similar to that previously determined for the gastrocnemius muscle (fig. 1). Overall, the mean increase in gracilis muscle $\dot{V}_{O_2}$ with SCh was slightly less than, but not significantly different from, that observed for the gastrocnemius muscle (table 3). For both, the increases in $\dot{V}_{O_2}$ were accomplished primarily by increases in extraction of O_2 rather than by increases in muscle blood flow.

With reduction in halothane from 1.7 to 0.1 per cent, $\dot{V}_{O_2}$ increased in each gracilis muscle studied. The mean change was small (10 per cent), significant, and similar to that observed for the gastrocnemius muscle (table 4). With the decrease in halothane, gracilis muscle blood flow increased approximately the same amount as did mean arterial blood pressure, which implies a lack of significant effect of halothane on gracilis muscle vascular resistance. A different response had been observed for the gastrocnemius muscle.

TABLE 3. Effects of Succinylcholine on Gracilis and Gastrocnemius Muscle $\dot{V}_{O_2}$ and $A\text{–}\dot{V}_{O_2}$

	Increase: Per Cent of Control					
	0–0.5 Hour		0.5–1.0 Hour		1.0–2.0 Hours	
	Mean	SE	Mean	SE	Mean	SE
Gracilis						
$\dot{V}_{O_2}$	35	4	19	3	12	3
$A\text{–}\dot{V}_{O_2}$	33	4	20	5	24	7
Gastrocnemius*						
$\dot{V}_{O_2}$	46	7	20	4	16	6
$A\text{–}\dot{V}_{O_2}$	34	6	17	9	14	12

* Previous study.[2]

TABLE 4. Gracilis and Gastrocnemius Muscle $\dot{V}_{O_2}$ and Blood Flow Values at 0.1 and 1.7 Per Cent Halothane, Expressed as Percentage Changes from 0.1 Per Cent Value*

	Gracilis (Per Cent)*		Gastrocnemius (Per Cent)†	
	Mean	SE	Mean	SE
$\dot{V}_{O_2}$	−10	2	−14	2
Blood flow	−25	7	19	4
Arterial pressure	−29	3	−29	3

* Present study, sequence 1.7 to 0.1 per cent halothane only.

† Previous study, sequence of concentrations alternated.[1]

Discussion

In the present studies, alterations in gracilis muscle $\dot{V}_{O_2}$ by halothane and SCh were approximately the same as alterations in gastrocnemius muscle $\dot{V}_{O_2}$ by the same drugs despite significant differences between these two muscles in terms of resting $\dot{V}_{O_2}$ *in vivo* and blood flow, composition, and $\dot{V}_{O_2}$ *in vitro*. Accordingly, there is some reassurance that it is appropriate to extrapolate the average responses of these two muscles to skeletal muscle generally and to prepare an estimate of

the halothane and SCh effects on whole-body $\dot{V}_{O_2}$. To do this, several assumptions are necessary. Skeletal muscle $\dot{V}_{O_2}$ will be assumed to comprise 35 per cent of whole-body $\dot{V}_{O_2}$. This is the approximate average of various indirect estimates for man,[4, 5] and the ratios of skeletal muscle mass to whole-body mass for dog and man are approximately the same (40 per cent).[14] Halothane will be assumed to decrease total skeletal muscle $\dot{V}_{O_2}$ 12 per cent, which is the average of the 10 and 14 per cent decreases observed for the gracilis and gastrocnemius muscles, respectively. Based on these assumptions, the decrease in whole-body $\dot{V}_{O_2}$ with halothane attributable solely to decrease in skeletal muscle $\dot{V}_{O_2}$ is approximately 4 per cent. Presumably, as was previously determined for the gastrocnemius muscle, this decrease occurs at 0.8 per cent halothane, without further reduction as halothane is progressively increased to 1.5 per cent.[1] The increase in muscle $\dot{V}_{O_2}$ with SCh had an initial peak phase which terminated within an hour, and a sustained phase after an hour. The average increases in $\dot{V}_{O_2}$ in the gracilis and gastrocnemius muscles during the first and second hours were 30 and 14 per cent, respectively. Accordingly, the 11 and 5 per cent increases in whole-body $\dot{V}_{O_2}$ during these times can be attributed to the increase in skeletal muscle $\dot{V}_{O_2}$ with SCh. The actual increases in whole-body $\dot{V}_{O_2}$ observed with SCh for these periods were 9 and 5 per cent, respectively.[15] This demonstration of good agreement between estimated and determined increases in $\dot{V}_{O_2}$ supports the appropriateness of the above assumptions, and, further, is consistent with the previous hypothesis that the entire increase in whole-body $\dot{V}_{O_2}$ with SCh can be attributed to the increase in skeletal muscle $\dot{V}_{O_2}$.[15]

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