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The effects of thermogenic agents on hindlimb oxygen consumption in the dog: ICI D7114 and noradrenaline

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Abstract. The thermogenic action of beta-adrenoceptor agonists may be due, in part, to increased metabolism in skeletal muscle. Previous results suggest that vasoconstriction is also necessary, and that the effect can be blocked by vasodilators. Both noradrenaline and the beta-3 agonist, ICI D7114, were studied using two dog hindlimb protocols. During constant perfusion conditions, ICI D7114 caused a significant increase in hindlimb oxygen consumption although it is a vasodilator. Noradrenaline resulted in a smaller rise in oxygen consumption, and produced a marked vasoconstriction. Both noradrenaline and ICI D7114 resulted in decreased oxygen consumption when the blood flow was allowed to vary in response to the drug treatment. The results suggest that changes in tissue oxygen consumption caused by beta-agonists are not related to vasomotion.
Key words. Beta-adrenoceptor agonist; skeletal muscle; thermogenic agents; oxygen consumption; noradrenaline.

Drugs which increase metabolic rate, in particular, thermogenic agents for the treatment of obesity, are frequently thought to stimulate the activity of brown adipose tissue (BAT). The induction of non-shivering thermogenesis (NST) by either cold-acclimation[1] or diet[2] has been attributed to the action of BAT. However, there are reports suggesting that skeletal muscle may also be a major thermogenic tissue[3–5].

In assessing the mechanism of action of thermogenic agents, it is useful to compare them with noradrenaline since it is considered to play a major role in whole body thermogenesis. There have been conflicting reports in the literature concerning the effect of noradrenaline on oxygen consumption by skeletal muscle. A number of workers have noted that noradrenaline increases oxygen consumption in the rat hindlimb. This has been summarized by Colquhoun et al.[3] and Ye et al.[6], who also show that oxygen consumption in the rat hindlimb can be increased by noradrenaline, that such an increase is flow-related, and that the effect is a vasoactive one (seen only with vasoconstriction). Research with cold-acclimatized animals also indicates that oxygen consumption is increased in skeletal muscle with infusion of noradrenaline, but to a somewhat lesser extent than in other tissues[7]. There are also reports in the literature, however, which suggest that noradrenaline, or sympathetic stimulation, causes a decreased oxygen extraction by skeletal muscle[8].

ICI D7114 is a novel beta-adrenoceptor agonist developed for the treatment of obesity and type 2 diabetes (non-insulin dependent). Administration of ICI D7114 to conscious rats results in marked increases in whole-body oxygen consumption as well as in the oxygen consumption of isolated BAT cells, and its structure and properties have been reported previously[9]. Since ICI D7114 is a vasodilator, a study was undertaken to determine whether or not it also increases oxygen consumption in skeletal muscle and to compare it to noradrenaline, which is known to cause a vasoconstriction. Such a comparison should help to clarify the effect of vasomotion resulting from the use of thermogenic agents. Two types of experiments were performed using the hindlimb of the dog: perfusion at constant flow rates, as well as perfusion when the blood flow rates were allowed to vary.

Materials and methods

Noradrenaline was obtained from the Sigma Chemical Co. Ltd., Poole, England. ICI D7114 was synthesized at ICI Pharmaceuticals, Alderley Park, England. Male and female beagle dogs, bred at ICI's laboratories were fed laboratory diet A (SDS Nutrition Ltd., Witham, England) and housed at 25 °C prior to use.

The animals were anesthetized with an intravenous bolus of pentobarbital sodium (30 mg/kg), followed by continuous infusion of the anesthetic at a level of 1.2 mg/min/kg for the duration of the experiment. After anesthesia was induced, the animals were intubated and ventilated with room air using a respiratory pump (Palmer). The left carotid artery was cannulated using PE-20 tubing for connection to a pressure transducer (Bell and Howell). The right jugular vein was also cannulated for use for later drug administration. The femoral artery and vein in the left hindlimb were cannulated using PE-20 tubing. These cannulae were inserted so that the tips were at the iliac bifurcation, as done by previous investigators[10], in order to obtain blood samples at the inlet and the outlet of the perfused limb. The placement was confirmed visually at the end of some experiments, and these cannulae were used to obtain the

arterial and venous samples for determination of the blood gases in the right hindlimb.

A Watson-Marlow peristaltic pump was used to control the blood flow rate to the right hindlimb in those experiments done under constant flow conditions. Male dogs were used for these experiments, and the influx for the pump was obtained by cannulating the femoral artery about 4–6 cm distally from the iliac bifurcation. The blood was returned to the same femoral artery by cannulating it about 5 cm distally from where it was removed. The saphenous artery was also cannulated, using PE-50 tubing which was inserted so that the tip was in the femoral artery, and connected to another pressure transducer in order to record hindlimb perfusion pressure. The pump was switched on and set at a constant blood flow rate so that the hindlimb pressure matched the mean blood pressure recorded from the carotid artery at the beginning of the control period. The right hindlimb was also denervated, by sectioning the sciatic and femoral nerves.

The experiments in which the blood flow to the right hindlimb was allowed to vary in response to the administration of a drug were done using essentially the same procedure, but with female dogs. In those experiments, no pump was inserted in the femoral artery. Instead, an ultrasound Doppler crystal was placed on the outside of the femoral artery in order to record the flow rates. The apparatus and recording devices were constructed by the ICI Pharmaceutical Laboratories for this purpose.

The experimental protocol was the same for all runs. First there was a 30-min interval, during which blood gases were determined every 10 min using a Nova STAT Profile Blood Gas Analyzer. Multiple readings on the same sample showed that this instrument gave excellent reproducibility of ± 1 mm Hg, and it was calibrated with standard gases often. The drug was administered via the jugular catheter, either as a bolus for ICI D7114 (1 mg/kg) or as an infusion for the noradrenaline (1 μg/min/kg). (Note: In one set of experiments, female dogs were dosed chronically (p.o.) with ICI D7114 contained in gelatin capsules, with the final dosage administered at the time of the experiment). The drugs used in the acute experiments were dissolved in isotonic saline. After the 30-min control period, blood gases were monitored for another 50–60 min. The heart rates were monitored using an ECG, and the temperature determined using a rectal thermometer. A heating blanket was placed under the animals to ensure that their temperatures were maintained at 38 ± 1 °C. At the end of the experimental period, the animals were euthanized while under terminal anesthesia. Oxygen consumption ($\dot{V}O_2$) was calculated as a product of the flow rate times the arterial-venous difference in oxygen content. (The oxygen content was determined by the Nova STAT Profile Blood Gas Analyzer since it also measures pH and hematocrit.)

Results

Constant flow. Table 1 lists the average values for overall body weight (W), hindlimb blood flow rates (Q), hindlimb oxygen consumption ($\dot{V}O_2$), and mean arterial blood pressure (BP) for 12 male dogs during the control period for each animal (the first 30 min). The values are listed as the mean $\pm$ the standard error of the mean. After a 30-min control period, the drug (or saline) was administered, and the oxygen consumption rates changed during the next 20 min until they reached a relatively constant value for the last 40 min of the experiment. It is this final change which is reported in table 1.

As seen in table 1, oxygen consumption appeared to increase slightly during the course of the experiment in the control (saline) group, but this was not statistically significant. The use of ICI D7114 resulted in a marked increased oxygen consumption as compared to the saline controls ($p < 0.05$). The mean arterial blood pressure fell with ICI D7114, indicating a vasodilatory effect. Noradrenaline, however, caused an increase in the mean blood pressure, indicating a vasoconstriction. Since the flow was constant for all experiments, the changes in blood pressure reflect a decrease in hemodynamic resistance with D7114 and an increase with noradrenaline. Noradrenaline also resulted in a slight increase in the oxygen consumption, but one which was not statistically different from the saline controls. It should also be noted that the circulatory hematocrit

Table 1. Constant flow experiments (male dogs)

Group	n	W (kg)	Q ($ml \cdot min^{-1} \cdot kg^{-1}$)	A–V diff ($ml\ O_2 \cdot ml\ blood^{-1}$)	$\dot{V}O_2$ ($ml \cdot min^{-1} \cdot kg^{-1}$)	BP (mm Hg)	HR ($beats \cdot min^{-1}$)
Untreated	12	14.9 ± 0.4	273 ± 14	2.3 ± 0.2	5.7 ± 0.4	144 ± 6	159 ± 5
				ΔA–V (%)	$\Delta\dot{V}O_2$ (%)	ΔBP (%)	ΔHR (%)
Saline, control	4	14.7 ± 0.9	273 ± 18	$+10 \pm 6\%$	$+13 \pm 10\%$	0	$-4 \pm 1\%$
ICI D7114	4	14.7 ± 0.3	277 ± 39	$+38 \pm 6\%$	$+41 \pm 10\%$[a]	$-11 \pm 4\%$	$+26 \pm 8\%$
Noradrenaline	4	15.2 ± 0.9	275 ± 36	$+20 \pm 14\%$	$+28 \pm 17\%$[b]	$+21 \pm 6\%$	$+12 \pm 6\%$

[a] $p < 0.05$, that value is different from saline control; [b] not statistically different from saline control.

Table 2. Constant flow experiments with chronically-treated female dogs

Group	n	W (kg)	Q ($ml \cdot min^{-1} \cdot kg^{-1}$)	$\dot{V}O_2$ ($ml \cdot min^{-1} \cdot kg^{-1}$)
Control	3	22.7 ± 3.3	270 ± 13	4.4 ± 0.5
ICI D7114	4	22.8 ± 1.4	273 ± 11	7.8 ± 1.6

Table 3. Free flow experiments (female dogs)

Group	n	W (kg)	Q ($ml \cdot min^{-1} \cdot kg^{-1}$)	A–V diff ($ml\ O_2 \cdot ml\ blood^{-1}$)	$\dot{V}O_2$ ($ml \cdot min^{-1} \cdot kg^{-1}$)	BP (mm Hg)	HR ($beats \cdot min^{-1}$)
Untreated	13	14.3 ± 0.3	193 ± 15	2.2 ± 01	3.8 ± 0.3	136 ± 4	163 ± 4
			ΔQ (%)	$\Delta A-V$ (%)	$\Delta \dot{V}O_2$ (%)	ΔBP (%)	ΔHR (%)
Saline, control	4	14.5 ± 0.6	$-13 \pm 3\%$	0	$-6 \pm 3\%$	0	0
ICI D7114	5	14.4 ± 0.2	a	$-17 \pm 4\%$	$-20 \pm 4\%$[b]	$-16 \pm 5\%$	$+10 \pm 4\%$
Noradrenaline	4	13.9 ± 0.6	$-72 \pm 4\%$	$-8 \pm 4\%$	$-68 \pm 3\%$[b]	$+20 \pm 3\%$	0

[a]Flow rate increased 52% in two animals; decreased 18% in three animals; [b]$p < 0.05$, that value is different from control.

increased in the dogs treated with noradrenaline by about 20% (it did not change with the other treatments).

An additional experiment was done with this same constant perfusion protocol using 7 female dogs. These animals had been dosed, orally, over a period of 6 weeks with either ICI D7114 or a placebo, and were, thus, somewhat larger. The results (table 2) show that resting hindlimb oxygen consumption rates for the female controls were similar to those for the males (4.4 ± 0.5 ml/min/kg vs 5.7 ± 0.5 ml/min/kg, n.s.). The effect of chronic dosing with ICI D7114 was similar to that seen when it was given as a single bolus, with hindlimb oxygen consumption increasing, and indicates that increased BAT (which resulted from chronic dosing with ICI D7114) may not be responsible for the effect on hindlimb oxygen consumption.

Variable flow. Another set of experiments was done in which the blood flow to the hindlimb was allowed to vary in response to the drug treatment. Table 3 shows the results from this experiment, and the mean oxygen consumption rate for the untreated dogs was quite similar to that found for the female dogs used in the previous experiments (3.8 vs 4.4 ml/min/kg, n.s.). During these variable flow experiments, the hindlimb oxygen consumption decreased slightly (6%) in the control (saline) experiments, probably as a result of a decreased (13%) blood flow to the hindlimb. Noradrenaline caused a large decrease in blood flow (72%) and a similar decrease in oxygen consumption. It also resulted in a 20% increase in mean blood pressure, the same change seen during the constant-flow protocol. (The circulatory hematocrit again increased with the use of noradrenaline, similar to the constant flow experiment.) ICI D7114 caused varied responses in the blood flow to the hindlimb. In two of the five dogs tested, the blood flow increased an average of 52%, while in three animals it decreased an average of 18%. These changes in flow did not correlate with changes in the blood pressure, which decreased in all animals receiving ICI D7114. Nor did it correlate with changes in heart rate caused by ICI D7114 (the heart rate inreased in all animals receiving ICI D7114 by an average of 10%). However, the hindlimb oxygen consumption did decrease more when the blood flow rate decreased than when it increased.

Discussion

Constant flow. The resting values for oxygen consumption in these animals are quite similar to those found previously in the dog hindlimb. From calculations using other data[11], it was determined that a value of 7–8 ml/min/kg is normal for the hindlimb oxygen consumption in unanesthetized dogs. In addition, the hindlimb blood flow rates in our animals were similar to those found previously by other investigators[11,12].

ICI D7114 increased hindlimb oxygen consumption markedly in this protocol. It also resulted in a vasodilatation of the peripheral blood vessels, as noted by a decrease in the mean arterial blood pressure as well as visual noting of the pink color it produced in the skin. Noradrenaline caused a marked vasoconstriction in the dog hindlimb, as evidenced by an increase in the mean arterial blood pressure (and the resistance), and a non-significant increase in oxygen consumption. This appears to differ from previous results (in the rat hindlimb) which suggest that vasoconstriction is necessary to cause increased oxygen consumption. Our results appear to indicate, instead, that vasomotion is not implicated in oxygen consumption changes.

Long-term dosing with D7114 showed the same effect as that found with its use in the acute experiments. Long-term dosing also appeared visually to result in the

formation of more BAT although there is relatively little of this tissue in the hindlimb. This suggests, however, that the increased oxygen consumption with ICI D7114 is not a result of a stimulation of brown adipose tissue since the effect was similar with both the acute and chronic dosing.

Noradrenaline caused an increase in circulating hematocrit of approximately 20%. Since this increases the blood oxygen concentration (similar to blood 'doping'), it might account for the effect on oxygen consumption. It has been noted previously that erythrocytes stored in the spleen of the dog are released upon sympathetic stimulation or administration of noradrenaline[13]. The spleen was not isolated in these experiments, and this was probably the cause of the increase in hematocrit seen during the 60-min duration of the experiments.

Variable flow. Experiments done under constant perfusion conditions are helpful in learning more about the mechanism of action of a particular drug on tissue oxygen consumption since the effect of one of the important parameters, flow, is eliminated. Consequently many studies of muscle oxygen consumption are done in this way. However, the in vivo action of a drug is perhaps better seen when the flow is allowed to vary naturally. Thus, a second set of experiments was performed in order to compare the effects of noradrenaline and ICI D7114 under such 'free flow' conditions. As shown in table 3, both NA and ICI D7114 resulted in decreased hindlimb oxygen consumption when the blood flow was allowed to vary in response to the drug. With noradrenaline, there was a large decrease in blood flow to the limb as well as a significant (68%) decrease in oxygen consumption. The decrease (around 20%) in oxygen consumption seen when ICI D7114 was given was less and the effect on flow was variable. Such flow changes did not correlate with the changes in blood pressure or heart rate, and may reflect a variability in the action of ICI D7114 in redistributing muscle blood flow. The calculated hemodynamic resistance with ICI D7114 showed no changes in three of the animals but a decrease of about 37% in the other two. The hemodynamic resistance increased by 400% with noradrenaline (it increased by 10% during the course of the control experiments).

With variable flow, both noradrenaline and ICI D7114 resulted in a decrease in hindlimb oxygen consumption although the effect was less marked for the ICI D7114. This may indicate that any thermogenic action of these compounds is not seen in skeletal muscle unless blood flow rates are maintained. Again, however, different vasomotion resulted in similar effects on oxygen consumption.

The results indicate that increased muscle oxygen consumption does not depend on a vasoconstricting effect, as has been suggested previously. When comparing the action of a vasodilator (ICI D7114) to that of a vasoconstrictor (noradrenaline), the hindlimb oxygen consumption was higher with the ICI D7114. These experiments used a protocol in which the hindlimb was perfused by the animal's own blood at normal temperatures and pressures, while non-erythrocyte perfusion fluids and lower pressures were used in some of the previous studies[3,6]. This may account for the different results seen.

It has also been suggested previously that ICI D7114 acts through another mechanism, the stimulation of brown adipose tissue. Adult dogs appear to possess relatively little of such tissue in their hindlimbs, although chronic dosing by ICI D7114 seems to stimulate its formation (as judged by visual observation). However, the results obtained in the acute and chronic experiments with ICI D7114 were quite similar, suggesting that the results are not related to stimulation of BAT.

Frequently, it is assumed that the oxygen consumption of a tissue is determined by the amount delivered to it, defined as the product of the blood flow rate times the oxygen content[16], at least up to a certain critical level. However, the amount delivered was quite similar for all animals in the constant-flow studies, which suggests that other factors may be important in determining tissue oxygen consumption. A recent series of papers[15–17] propose that diffusion of oxygen from the erythrocytes to the mitochondria is controlling when the oxygen consumption rate is maximal, and perhaps this is also important at resting conditions. It appears that ICI D7114 results in a redistribution of blood flow to the tissue, and this might result in decreased diffusion distances and an increase in the oxygen consumption rate as long as perfusion is maintained (as in the constant flow experiments). In any event, though, changes in oxygen consumption in the hindlimb can be similar with both vasodilating and vasoconstricting thermogenic agents.

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