Regulation of shivering and non-shivering heat production during acclimation of rats

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Davis, T. R. A., D. R. Johnston, F. C. Bell and B. J. Cremer. Regulation of shivering and nonshivering heat production during acclimation of rats. Am. J. Physiol. 198(3): 471-475. 1960.-When cold acclimating rats are treated with diathermy, curare and a combination of both, two main fractions of the increase in cold-induced oxygen consumption can be delineated. First, a fraction which diathermy replaces by virtue of the fact that it, in the intensities used, can raise core temperature without altering the temperature of the skin; therefore this fraction appears to be dependent upon changes in central temperature and is found to persist throughout the period of acclimation investigated. Second, a fraction of cold-induced oxygen consumption which is not replaced by diathermy and which is presumed to be dependent upon changes in skin temperature. By the administration of curare, this second fraction can be separated into two further fractions acting reciprocally depending upon the duration of cold exposure. In the early stages of acclimation, the curare-suppressed fraction of oxygen consumption appears to be entirely due to shivering. As shivering disappears with acclimation, it is replaced by a peripherally regulated nonshivering heat source which eventually takes over all the duties of heat production previously performed by shivering.

Using curare, Claude Bernard (1) in 1876 reported data which he interpreted as indicating that nonshivering thermogenesis existed in addition to shivering heat production. Rubner in 1902 (2), Lelevre in 1911 (3), and Cannon in 1927 (4) agreed that there may be a nonshivering heat production in addition to that produced by shivering. In 1954 Sellers (5) showed that in spite of a maintained high oxygen consumption in the cold, shivering was less in the acclimated than in the nonacclimated rat. In 1956 Heroux et al. (6) using electromyographic recordings, and Cottle and Carlson (7) using curare, substantiated the disappearance of shivering during acclimation. In 1954 Davis and Mayer (8), using the techniques of curare and diathermy (9) in the nonacclimated rat, interpreted their findings as substantiating the fact that a nonshivering thermogenesis exists in the presence of shivering. From their results (8, 10) they further deduced that shivering was primarily stimulated by changes in skin temperature and that the oxygen consumption resulting from nonshivering sources was sensitive to changes of central body temperature. This dual regulation, based upon changing temperatures of both skin and brain, was first proposed by Richet in 1898 (11).

This report presents data which indicate that a dual regulation exists in both the acclimated and nonacclimated rat. In the nonacclimated rat, this dual regulation is represented by both shivering and nonshivering heat production as previously reported (8) and, in the acclimated rat, entirely by nonshivering heat production.

METHODS

This study utilized 200 Sprague-Dawley rats ranging from 225 to 280 gm in weight. The rats were warm-acclimated at a temperature of 28°C for 3–6 weeks prior to cold acclimation in single cages without bedding in environmental temperatures of 13°C, 6°C and 2°C. Water and food were given ad libitum.

Shivering was measured by integrating the electromyographically obtained action muscle potentials as described in a previous report (10). The integrated signals were continuously recorded with a Sanborn Twin-Viso recorder and the electrical activity of shivering was measured in volts per minute. All temperatures were measured with a 16-channel Brown potentiometer utilizing copper constantan thermocouples. Diathermy at a frequency of 27.12 megacycles/sec. was produced by a radio frequency generator previously described (9) and a Burdick MF 49 diathermy machine.
Oxygen consumption was obtained in fasted animals (16-20 hr. in the warm and 8-12 hr. in the cold) by the closed circuit system, the principles of which have already been described (8). This method produced results which were not materially different from those obtained by the Haldane open circuit method (9); it was chosen for greater simplicity and also because continuous recordings could be obtained.

The intraperitoneal dosage of d-tubocurarine used was 0.0170 mg/100 gm of rat. It was found that in order to achieve certain criteria this dosage had to be adjusted to the third and fourth decimal points by stepping the dosage up or down as little as 0.0005 mg or as much as 0.0015 mg. Thus extra animals had to be included in each group to act as dosage adjusters. The criteria of correct dosage were: a) the animal must collapse physically and be unable to stand for a period of not less than 6 minutes and no greater than 10 minutes thus insuring that hypothermia did not constitute an extra variable. Before and after measurements of both rectal temperature and oxygen consumption did not show the existence of a significant hypothermia. b) The animal lived after curarization. c) The curarization stopped shivering as indicated by electromyographic recordings. d) The dosage used was at a level which was unlikely to produce central nervous system effects (12).

Cold room temperatures were maintained at 13°C, 6°C and 2°C but all measurements of shivering were performed at 6°C and all oxygen consumptions were obtained with chamber temperatures varying between 9°C and 11°C.

Oxygen uptake in vitro of muscle tissue (quadratus femoris) after sacrifice was determined by the Warburg technique run at 37°C. The rats used in this experiment were exposed to 6°C and sacrificed at intervals during the period of acclimation. Four samples from each of 10 rats were used to obtain data at each interval.

RESULTS

The effect of dielectric diathermic warming on rectal temperature, skin temperature and cold-induced oxygen consumption expressed as percentage change is represented in figure 1 where diathermy was applied for 10 minutes at 10-watt increments. Above 40 watts input to the final stage of the radio frequency generator, rectal temperature rises as the diathermic field intensity increases, however, skin temperatures remain unaffected until the field intensity reaches values above 100 watts, at which point it rises sharply. The cold-induced oxygen consumption remains at the initial level until an intensity of 40 watts is reached, where it falls approximately 45%. It remains at this level with increase of diathermic intensities until reaching 115 watts when it falls towards basal oxygen consumption levels accompanied by an increase in skin temperature and, in spite of the continued application of diathermy, a fall in rectal temperature. Therefore, at intensities between 50 and 100 watts and under the experimental conditions, diathermy appears to separate the cold-induced heat production which is stimulated by changes in central temperature from the heat production which is stimulated by changes in skin temperature. A range of 50-70 watts was chosen for the experiment because this range produced the limit of depression of cold-induced oxygen consumption. It produced no measurable effect upon skin temperature and did not produce an excessive rise in central temperature. The method of diathermic application has been previously described (8).

Figure 2 shows the changes in the electrical activity of shivering and cold-induced oxygen consumption of groups of animals exposed to environmental temperatures of 13°C, 6°C and 2°C, 12 animals being used in each group. Changes in shivering activity of the 13°C and 6°C group show a marked fall for both exposure temperatures. In both instances near-zero activity was achieved. There is some delay in the disappearance of shivering in the 13°C group as compared with that of the 6°C group, otherwise the curves shown no real differences. After an initial rise, the oxygen consumption values for the two groups show a downward trend which is significantly greater in the 13°C group than in the 6°C group. The oxygen consumption curve for the 2°C group shows an upward trend after 13 days of exposure and the curve for the 13°C group shows a similar upward trend after 35 days of exposure. In all groups the cold-induced oxygen consumption does not fall to basal levels, whereas shivering disappeared altogether in the 6°C and 13°C groups. Lack of appropriate facilities did not allow shivering activity to be measured in the group exposed to 2°C ambient air temperature.
Figure 2. Influence of continued cold exposure upon shivering and cold-induced oxygen consumption (12 rats in each group).

Figure 3 shows oxygen consumptions expressed as percentage change of cold-induced oxygen consumption obtained at different environmental temperatures (13°C, 6°C and 2°C) when animals are treated with diathermy. As previously reported, diathermy reduces the above basal oxygen consumption in unacclimated rats and mice by approximately 45% (9, 10). As exposure continues there is a general downward trend in the oxygen consumption curves until a low is reached in each case. In the 2°C group a considerable instability was observed near this low point and deaths, when they occurred, did so during this period; these lows occur at 12 days for the 2°C group, at 21 days for the 6°C group, and at 38 days for the 13°C group. This downward trend occurred less abruptly than the fall in shivering activity. In each group, after this low has been reached, a marked upward trend in the oxygen consumption of the diathermy-treated animals occurs, during which period little shivering can be demonstrated. Thus diathermy lowers oxygen consumption in the cold to a varying extent depending upon the duration of exposure to cold.

Figure 4 shows curves of oxygen consumption in the cold obtained when the animals are treated with curare in the one instance and with curare and diathermy in the other, both curves being expressed as percentage of the cold-induced oxygen consumption. The differences between the points in the two curves are significant at the 1% level of confidence, but for all intents and purposes the two curves are parallel. Diathermy has depressed the curare curve by approximately 40–50% at all points (a different group of animals was used for each point in the two experiments represented in this figure).

Figure 5 depicts the oxygen uptake of muscle tissue as determined by the Warburg technique. Each point represents the mean of data obtained on four samples from each of 10 animals, sacrificed at the time intervals indicated. Muscle oxygen uptake is significantly raised on the third day and the values obtained on the 13th day and thereafter are significantly increased as compared with the values obtained from controls on zero day and also from the 5th day samples.

Discussion

As already demonstrated by Sellers (5), Heroux (6) and Cottle (7), shivering disappears as a result of acclimation. Comparable diminution of total oxygen consumption in the cold does not occur even though a marked fall in total oxygen consumption is perceived 35 days after exposure to 13°C. However, there is a tendency for oxygen consumption to rise after this period in spite of the fact that shivering cannot be demonstrated. Sellers (5) has already shown that the oxygen consumption between 42 and 90 days of cold acclimation at 2°C is higher than that of unacclimated rats and, since shivering disappears after 25 days, it may be presumed that this disappearance is perhaps only the beginning of the process of true acclimation.

Diathermy lowers the cold-induced oxygen consumption presumably by causing an increase in core temperature and thus decreasing the need for a metabolic source of heat production. The oxygen consumption does not return to warm room basal values because dia-
The failure of diathermy to raise skin temperature may be explained in several ways: a) the intensity required to alter central temperature is insufficient to overcome heat loss at the skin surface. b) Vasoconstriction is a biologically active mechanism which is responding to the ambient air temperature that, being a dielectric, is not altered thermally by radio frequency waves. c) It can be demonstrated that, in the cold, diathermy at a frequency in the neighborhood of 27.12 megacycles can raise the central temperature of a dead rat to a greater extent than it can the surface temperature. This property is probably associated with the penetrating power of radio waves near this frequency as opposed to microwaves at frequencies around 2400 megacycles where surface temperatures are affected to a greater extent than deeper temperatures (13). Although the proportion of the surface of the rat which is not furred is small, it is possible that the number of cold sensitive receptors in this area may be proportionately greater, moreover previous work has shown that merely insulating the tail of a rat significantly alters the rate of increase in shivering activity (10).

In all of the groups treated with diathermy, two phases, separated by a low point, can be distinguished in the oxygen consumption curves (fig. 3). The downward trend in the first phase parallels the decrease of shivering activity, but correlates with shivering poorly (+0.5). Furthermore, the second phase of the curve shows an upward trend which occurs when shivering is minimal. In a previous report it was shown that in the unacclimated rat the part of oxygen consumption which could not be replaced by diathermy could be eliminated by curare or by raising skin temperature and was therefore due primarily to shivering (8). Accordingly it would appear that shivering in the non-acclimated animal is the major source of peripherally regulated heat production. As exposure increases, the curve of oxygen consumption in the diathermized rat re-establishes itself without the presence of shivering, therefore, peripherally regulated heat production now appears to arise from nonshivering sources and takes over the duties ascribed to shivering in the nonacclimated animal. Further support of this is available in the oxygen consumption curves obtained in the diather-
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mized and nondiathermized animals treated with curare (fig. 4) confirming the findings of Cottle and Carlson (7). Because curare eliminates shivering, both curves represent the oxygen consumption not due to shivering and both, negatively and significantly, correlate with the fall in shivering obtained by the integrated EMG recordings represented in figure 2. Thus the metabolic cost of shivering can be obtained either by subtracting the curare oxygen measurements from total oxygen consumption or by subtracting the oxygen measurements of curare and diathermy treated animals from the oxygen consumptions of animals treated with diathermy alone. The oxygen consumption curve in the diathermized rat treated with curare can reasonably be presumed to measure the appearance of peripherally stimulated nonshivering heat production.

Our data summarized graphically in figure 6, indicates that the cold-induced oxygen consumption represented by the upper graph is the algebraic sum of three mechanisms of oxygen consumption: a) a peripherally stimulated oxygen consumption due to shivering and abolished by curare; b) a nonshivering peripherally stimulated oxygen consumption, unaffected by curare or diathermy and possibly due to an increase in tissue metabolism (fig. 5); c) a centrally stimulated nonshivering oxygen consumption which can be abolished by diathermy and is unaffected by acclimation.

In the unacclimated and the fully acclimated rat, the proportion of peripherally and centrally regulated cold-induced heat production is approximately 1:1. At any moment during the process of acclimation, peripherally induced heat production is shared reciprocally by shivering and nonshivering sources of metabolism, depending upon the degree of acclimation. These findings, however, do not exclude the possibility of centrally stimulated shivering occurring under different circumstances. Two of these circumstances, namely hyperthermia and hypothermia of a sufficient degree, have already been pointed out in a previous report (10).

The fact that muscle tissue in vitro shows an increased ability to take up oxygen during the period of exposure when shivering was not demonstrable, indicates that muscle may be able to produce heat in the noncontractile state during cold exposure. Since curare does not affect the oxygen consumption of the unacclimated rat, one must presume that if noncontracting muscle does indeed produce heat for thermoregulatory purposes, neural pathways, if such are necessary for its stimulation and regulation, must not be via the neuromuscular junction; alternatively we may presume that this type of heat production is humorally mediated, and since the techniques of curare and diathermy indicate that the centrally stimulated fraction of cold-induced metabolism is unchanged by acclimation, it is possible that the peripherally stimulated nonshivering fraction which replaces shivering may be produced in part by a noncontractile type of muscular heat production.

If these results and the results of Bernard (1), Sellers (5), Davis (8), Heroux (6) and Cottle (7) are correctly interpreted, nonshivering temperature regulation plays an important role in cold exposure, being partly responsible for heat production in the unacclimated rat and totally responsible for heat production in the acclimated animal. At the present stage of knowledge the source or sources of nonshivering thermogenesis can only be hypotheses. The data of Hill (14) on heat production of muscle with no mechanical response and the work of Hsieh (15) demonstrating that the calorigenic effect of norepinephrine is significantly increased in acclimatized animals, both give some indication of the possible sources of nonshivering heat production during cold exposure.

REFERENCES

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