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EFFECTS OF NORADRENALINE AND CARBACHOL ON TEMPERATURE REGULATION OF COLD-STRESSED AND COLD-ACCLIMATED RATS

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1 Noradrenaline (20 μg) and carbachol (1 μg) injected into the anterior hypothalamus of rats at an ambient temperature of 23°C evoked significant falls in core temperature and increases in tail temperature.

2 When rats were cold-stressed (4°C for 90 min) or cold-acclimated (4°C for 4 weeks) and the above amine injections repeated, only carbachol evoked significant falls in core temperature and neither amine increased tail temperature.

3 Central injections of noradrenaline and carbachol also evoked increases in plasma glucose concentrations but not plasma non-esterified fatty acid (NEFA) concentrations in control, acutely cold-stressed and cold-acclimated rats.

4 Although concentrations of plasma glucose and blood lactate of rats were unaffected by cold exposure to 4°C for 1 to 28 days, glucose oxidation rate of both cold-stressed and cold-acclimated rats was significantly greater than in rats at 23°C. Concentrations of plasma NEFA were increased after 1 to 28 days of cold exposure.

Introduction

Hypothalamic centres which are sensitive to injections of noradrenaline, acetylcholine and other amines regulate the body temperature of homeotherms. When a homeotherm is exposed to cold, these centres oppose the rapid loss of heat by increasing sympathetic tone (to produce pilo-erection and peripheral vasoconstriction) and by increasing metabolic rate. Increased metabolic rate is secondary to increased motor activity and shivering thermogenesis, responses which are mediated by somatic nerves (Thompson, 1977). If an animal is exposed chronically to cold for 3 to 4 weeks it adapts (acclimates) to the cold-stress principally by replacing shivering thermogenesis with non-shivering thermogenesis (Sellers, Scott & Thomas, 1954). Whereas shivering is confined to skeletal muscle and is mediated by somatic nerves, non-shivering thermogenesis occurs principally in brown adipose tissue (Foster & Frydman, 1978) and is mediated by the sympathetic division of the autonomic nervous system (Hsieh & Carlson, 1957; Seydoux & Giradier, 1977). Nevertheless, the energy for each is derived from the mobilization and burning of free fatty acids (FFA) and glucose (Himms-Hagen, 1972; Thompson, 1977). Rats adapt readily to cold stress (usually 4°C) and are therefore commonly used in studies of the metabolic effects of acute cold stress and cold acclimation.

Neuronal models have been formulated to explain the central effects of amines on thermoregulation, e.g. that of Bligh, Cottle & Maskrey (1971) to explain the effects of intracerebroventricular injections of noradrenaline, 5-hydroxytryptamine and acetylcholine on the temperature regulation of sheep, goats and rabbits above, at and below thermoneutrality. Apart from one paper on noradrenaline (Schmidt, 1963), no comparable study exists for the rat. Moreover, no studies of the effects of central injections of amines exist for cold-acclimated animals. The purpose of the present investigation was to compare the effects of noradrenaline and carbachol on the temperature regulation of rats at a thermoneutral ambient temperature (23°C: Poole & Stephenson, 1977a) and during acute (90 min) and chronic (4 weeks) exposure to cold (4°C). The injections were made into the anterior hypothalamus because in a previous study (Poole & Stephenson, 1979) noradrenaline and carbachol injections into this region had pronounced effects on temperature regulation with short latencies to onset. Body temperature, tail temperature (an index of heat loss), blood lactate and plasma concentrations of free fatty

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acids (FFA) and glucose were monitored in conscious, unrestrained rats after injections of these amines under the three conditions defined.

Methods

The rats used in this study were male Wistar ($n = 160$), housed singly in plastic cages (335 mm × 210 mm × 170 mm) at 23 ± 1.5°C with water and food (Diet 41B, Dixons Foods Ltd.) *ad libitum*, and subject to a 12 h light (06h 00min to 18h 00min)/dark (18h 00min to 06h 00min) cycle.

Materials

Thermistor probes were constructed as described previously (Poole & Stephenson, 1977b). Intravenous cannulae were constructed from 100 mm lengths of silicone rubber tubing (Silastic 602-155, i.d. 0.6 mm, o.d. 1.2 mm, Dow Corning Ltd.) with a spherical silicone rubber (Silastic 734 RTV, Dow Corning Ltd.) collar, 3 mm in diameter, 25 mm from the tip. Each cannula was filled with pyrogen-free sterile saline (0.9% w/v NaCl solution) and its tip blocked with a small plug of paraffin wax (m.p. 45°C, Hopkin & Williams Ltd.) Caps for the cannulae were constructed from 21-gauge stainless-steel hypodermic needles closed with a silicone rubber plug through which injections could be made.

Operative procedures

One hundred rats (275 to 325 g) were anaesthetized with halothane and, under aseptic conditions, a 22-gauge stainless-steel guide cannula with stylette was stereotactically implanted into the left or right anterior hypothalamus at co-ordinates derived from the atlases of Fifková & Maršala (1967) and König & Klippel (1963). The co-ordinates were 0.2 mm posterior to bregma, 0.5 mm lateral to the midline and at a depth of 8.5 mm below the surface of the skull. After further growth to 325 to 350 g (about 2 weeks), 15 of these rats were anaesthetized with halothane and thermistor probes implanted into their thoracic cavities (Poole & Stephenson, 1977b).

A further 15 rats (275 to 325 g) were anaesthetized with halothane and their skulls implanted chronically with screws and dental cement (Poole & Stephenson, 1977a). Under aseptic conditions, a 30 mm incision was made along the midline of the throat and upper thorax and an intravenous cannula inserted 25 mm into the right jugular vein so that its blocked tip lay close to the heart. The cannula was secured by ligatures either side of its collar and the free end drawn subcutaneously to the skull, exteriorized immediately behind the existing implant and attached to the cap. The incision in the throat was sutured and the cap attached with acrylic cement to the implant on the skull.

All implanted rats were housed at 23 ± 1.5°C for 2 weeks after surgery and then at either 23 ± 1.5°C or 4 ± 0.5°C.

Experimental procedures: body temperature determinations

Rats implanted with hypothalamic guide cannulae and intrathoracic thermistor probes were placed singly in a perspex environmental chamber (335 mm × 290 mm × 280 mm) at 23 ± 0.5°C or 4 ± 0.5°C, through which air flowed at 2 litres/min. The thoracic probe and a second thermistor (of diameter 2.5 mm) taped to the dorsal surface of the tail, 20 mm from its base, were connected to a Devices pen recorder via a microcable (Radiospares Ltd.), a mercury concentric swivel (Campden Instruments Ltd.) and bridge circuits (Allen & Lanworn, 1968). For central injections a 27-gauge stainless-steel cannula, connected by polyethylene tubing (Portex PP20, Portland Plastics Ltd.) to a 10 µl syringe, was inserted so that its tip was 0.2 mm below the tip of the guide cannula. The inner cannula contained pyrogen-free sterile saline for control injections or one of the following drugs in sterile saline: (−)-noradrenaline hydrochloride (20 mg/ml, Sigma) or carbamylcholine (carbachol) chloride (1 mg/ml, BDH). The pH of all injected solutions was between 4 and 5 and the osmotic pressures of the injected solutions were saline: 3.08×10^{-4} milliosmol/µl, noradrenaline: 5.32×10^{-4} milliosmol/µl and carbachol: 3.22×10^{-4} milliosmol/µl. When core temperature and tail temperature had been monitored for 90 min, during which time body temperature had stabilized (Poole & Stephenson, 1977b), 1 µl of saline, noradrenaline, or carbachol was injected (over 1 s) and the resulting temperature changes recorded. After temperature responses to intrahypothalamic injections of noradrenaline and carbachol had been obtained at 23 ± 0.5°C and 4 ± 0.5°C, the rats were housed at 4 ± 0.5°C for 28 days and the experiments were repeated at this ambient temperature. At least 1 week elapsed between experiments in the same rat and no animal was given more than 4 injections.

Plasma nutrients and body weight determinations

Rats implanted with hypothalamic guide cannulae were placed singly in the experimental chamber at 23 ± 0.5°C or 4 ± 0.5°C. For central injections the inner cannula was inserted as before and 90 min elapsed before an animal was given an injection. At intervals of 0, 5, 15 and 30 min after injection rats were killed by a blow on the head and bled via the carotid arteries. The blood was collected, immediately

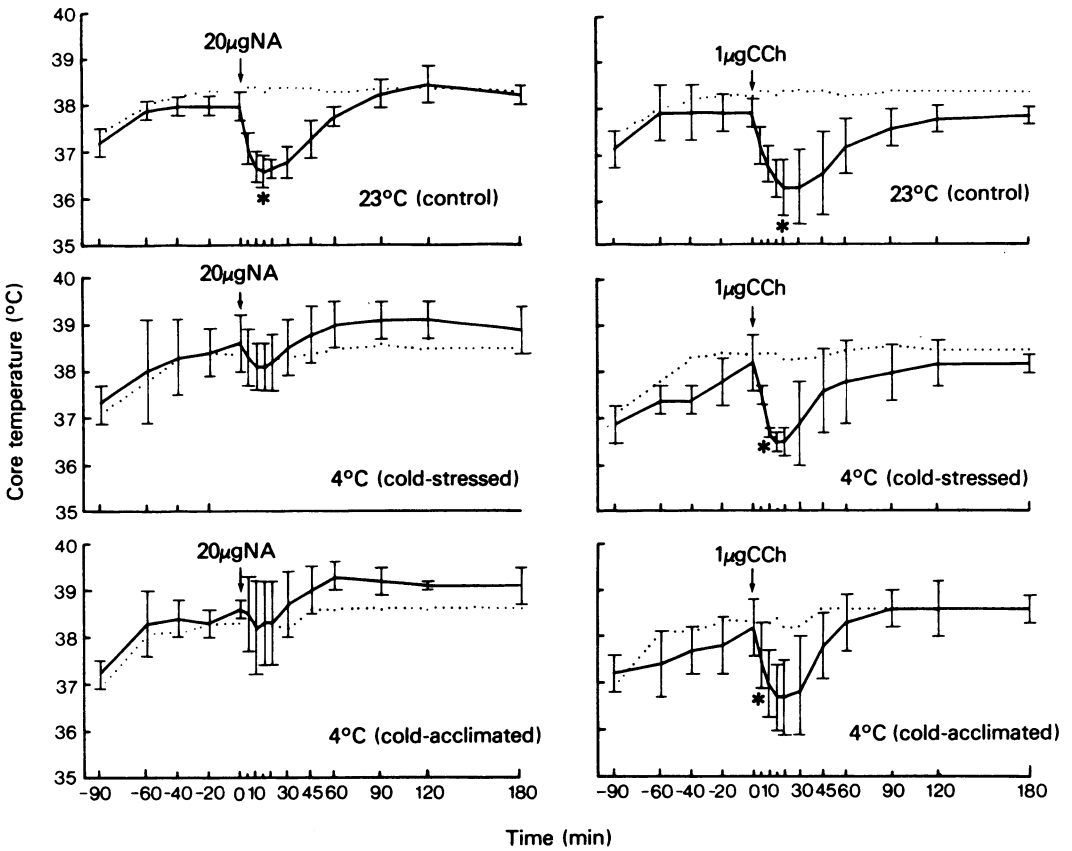

Figure 1 The effects of intrahypothalamic injections of saline (dotted line), noradrenaline (NA, 20 μg) and carbachol (CCh, 1 μg) on the core temperatures (mean ± s.d., $n \geq 5$) of control, acutely cold-stressed and cold-acclimated rats. $^{*}P < 0.001$.

centrifuged (2300 *g* for 15 min) and the plasma assayed for non-esterified fatty acids (NEFA) and glucose as described previously (Marley & Stephenson, 1975).

Forty-five rats, housed at 23 ± 1.5°C, were divided into 5 groups each of 9 rats, the mean weights of each group being approximately equal. Nine days later the groups were weighed and then separated: 1 group remained at 23 ± 1.5°C, 3 groups were placed at 4 ± 0.5°C and the 5th group was killed, bled and concentrations of plasma NEFA, glucose and blood lactate determined (Marley & Stephenson, 1975). The group housed at 23 ± 1.5°C and one of the groups living at 4 ± 0.5°C were weighed after 1, 7, 14, 21 and 28 days of cold acclimation, killed on the 28th day, bled and concentrations of plasma NEFA, glucose and blood lactate determined. The 2 other groups living at 4 ± 0.5°C were killed after 1 and 7 days of cold acclimation, respectively, and their blood/plasma was likewise assayed.

Determination of glucose oxidation rate

Rats implanted chronically with intravenous cannulae were placed singly in a glass experimental chamber (120 mm diameter, 210 mm high) at 23 ± 0.5°C or 4 ± 0.5°C, through which dry, CO_2-free air flowed at 2 litres/min. For intravenous injections a 27-gauge stainless-steel cannula was inserted below the silicone rubber plug on the skull. The cannula contained uniformly labelled D-[^{14}C]-glucose (5 μCi in 0.1 ml/100 g) and was connected, via an air-tight seal in the top of the chamber, to a 1 ml syringe with polyethylene tubing (Portex PP20, Portaland Plastics Ltd). When a rat had been in the chamber for 90 min the glucose solution was injected (over 2 s) and washed in with 0.2 ml pyrogen-free sterile saline. The air leaving the chamber was passed through a tube containing $CaCl_2$, into a 1 litre Cary-Tolbert ionisation chamber and finally to a CO_2 analyser (Hartmann & Braun Ltd.) The β radioactivity from the $^{14}CO_2$

was measured with a vibrating reed electrometer (Cary 410M, Varian Ltd) and displayed, together with total CO_2 output, on a potentiometric recorder. The electrometer-ion chamber unit was calibrated with nitrogen containing 1 μCi $^{14}CO_2$/litre.

Results

Noradrenaline (20 μg) and carbachol (1 μg) injected into the anterior hypothalamus of rats at 23°C evoked significant falls ($P < 0.001$) in core temperature and increases ($P < 0.001$) in tail temperature (Figures 1 and 5). In acutely cold-stressed (4°C) rats the mean fall in core temperature after carbachol injection was similar ($P > 0.05$) to the hypothermia evoked at 23°C (Figures 1 and 5). In contrast, the hypothermic response to noradrenaline was reduced by 47%, to a fall which was not statistically significant. The amine injections were repeated at 4°C after cold-acclimation and the induced falls in core temperature were shown to be similar ($P > 0.05$) to those evoked in cold-stressed rats (Figures 1 and 5). No significant increases in tail temperature were evoked by noradrenaline and carbachol in cold-stressed or cold-acclimated animals (Figure 5). The delayed increases in core temperature observed after noradrenaline injections in cold-stressed and cold-acclimated rats were not statistically significant ($P > 0.05$).

The effects of cold exposure to 4°C for up to 28 days on the body weights of 9 rats is shown in Figure 2. The small (3%) decrease in the mean body weight after cold-exposure for 1 day was not statistically significant ($P > 0.05$). However, the mean body weight of cold-exposed rats increased more slowly than that of a control group of 9 rats housed at 23°C and differences were significant (P values ranged from $P < 0.01$ to $P < 0.001$) after 7, 14, 21 and 28 days of cold exposure.

Although concentrations of plasma glucose and blood lactate of rats (determined immediately after removal from their home cages) were unaffected by cold-exposure to 4°C for 1, 7 and 28 days (Table 1).

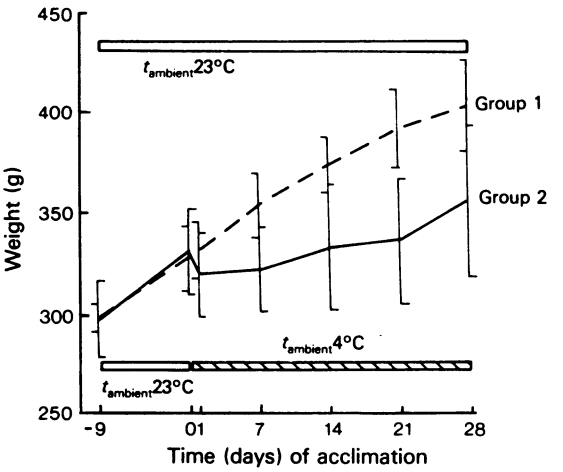

Figure 2 The effects of cold exposure to 4°C for up to 28 days on the body weights (mean ± s.d.) of 9 rats (Group 2), relative to a control group (1) of 9 rats at 23°C. The mean body weight of the control group was greater (P values ranged from $P < 0.01$ to $P < 0.001$) after 7, 14, 21 and 28 days.

glucose oxidation rate of both cold-stressed and cold-acclimated rats was significantly greater ($P < 0.01$) than in rats at 23°C. From Figure 3 it can be seen that the peak specific activity of expired CO_2 was reached 44 ± 11 min after injection of [^{14}C]-glucose at 23°C, whereas in cold-stressed and cold-acclimated rats peak specific activity occurred at 23 ± 3 min and 19 ± 6 min, respectively after injection. This reduction was significant ($P < 0.01$), as was the reduction in the mean $T_{\frac{1}{2}}$ of injected [^{14}C]-glucose from 254 ± 102 min in rats at 23°C to 62 ± 21 min in cold-stressed rats and 55 ± 12 min in cold-acclimated animals ($P < 0.01$). At 23°C, rats displayed periods of motor activity or grooming alternating with inactivity and sleep; these variations in behaviour gave rise to a large standard deviation of the mean $T_{\frac{1}{2}}$ of injected [^{14}C]-glucose. The behaviour of cold-stressed

Table 1 The effects of cold exposure to 4°C for up to 28 days on the body weights and concentrations of plasma nutrients (mean ± s.d.) of 4 groups each of 9 rats

	No. of days of cold exposure			
	0	*1*	*7*	*28*
Body weight (g)	330 ± 21	319 ± 21	321 ± 21	355 ± 38
Plasma NEFA (μEq/l)	124 ± 14	143 ± 25	153 ± 24*	133 ± 19
Plasma glucose (mg%)	128 ± 7	129 ± 10	134 ± 7	130 ± 7
Blood lactate (mg%)	17 ± 3	16 ± 5	21 ± 7	17 ± 3

The mean increase in plasma NEFA concentrations at 7 days (*) was statistically significant ($P < 0.005$).

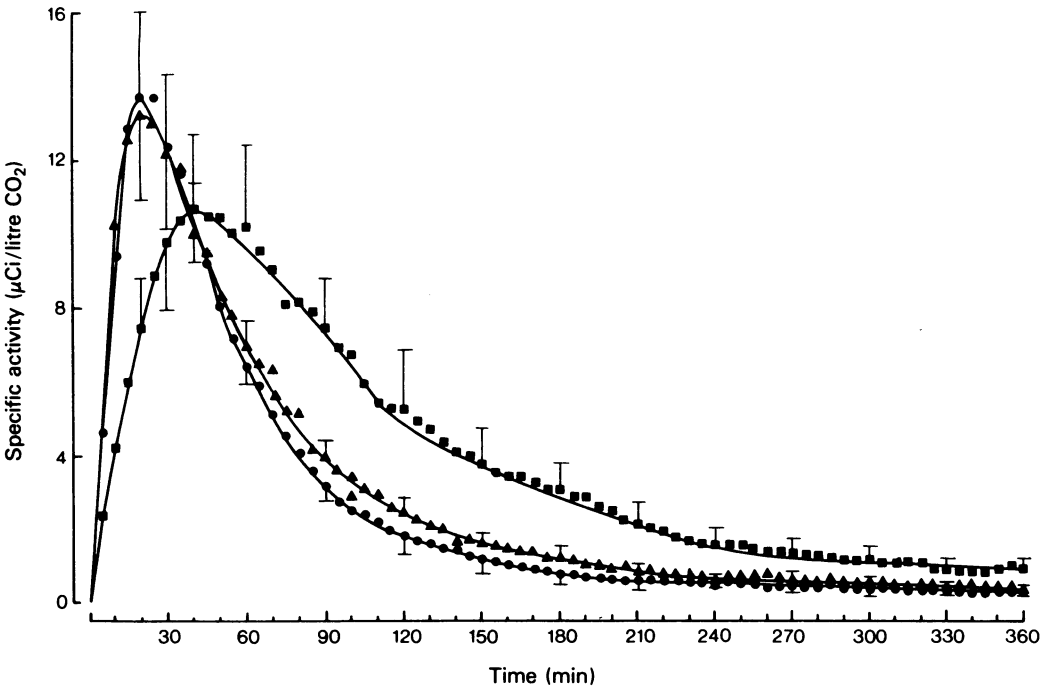

Figure 3 The specific activity of CO_2 expired from control, acutely cold-stressed and cold-acclimated rats ($n = 5$) after intravenous injections of uniformly labelled D-[^{14}C]-glucose (5 μCi in 0.1 ml/100 g). The symbols represent the means of 5 experiments and the vertical bars are standard deviations of the means: (■) 23°C (control); (●) 4°C (cold-stressed); (▲) 4°C (cold-acclimated).

and cold-acclimated rats was more consistent, cold-stressed rats being the most active. Concentrations of plasma NEFA were increased after 1, 7 and 28 days of cold exposure (Table 1) although the increases were only significant ($P < 0.005$) at 7 days (Table 1).

Control plasma glucose levels (i.e. after saline injection) were similar ($P > 0.05$) in normal, cold-stressed and cold-acclimated rats. Noradrenaline (20 μg) and carbachol (1 μg) injected into the anterior hypothalamus of rats at 23°C evoked significant increases (P values ranged from $P < 0.025$ to $P < 0.005$) in plasma glucose concentrations measured 5, 15 and 30 min after injection (Figure 4). The greatest mean increase evoked by noradrenaline was 54%, measured 15 min after injection, declining to 22% at 30 min, whereas carbachol evoked increases of 50% and 55%, measured 15 and 30 min respectively after injection. Changes in plasma NEFA concentrations were not significant. A time interval of 15 min after amine injection was therefore selected for subsequent determinations of the effects of noradrenaline and carbachol on plasma NEFA and glucose concentrations. The increases in plasma glucose and NEFA measured 15 min after intrahypothalamic injections of saline, noradrenaline (20 μg) and carbachol (1 μg) in normal (23°C), cold-stressed (4°C for 90 min) and cold-acclimated (4°C for 28 days) rats are shown in Figure 5. Noradrenaline and carbachol injections significantly increased (P values ranged from $P < 0.01$ to $P < 0.001$) plasma glucose concentrations, the increases evoked by noradrenaline being similar (38 to 44%) under all three conditions. In contrast, the increases of 97 and 77% evoked by carbachol in normal and cold-stressed rats, respectively, were significantly greater ($P < 0.001$ and $P < 0.05$) than the increase of 44% evoked in cold-acclimated animals. Although amine-induced increases in plasma NEFA were not significant, the control NEFA levels (i.e. after saline injection) were more than 100% greater in cold-stressed ($P < 0.02$) and cold-acclimated rats than in rats at 23°C (Figure 5).

Discussion

Noradrenaline and carbachol evoked substantial falls in core temperature and increases in tail temperature when injected into the anterior hypothalamus of rats

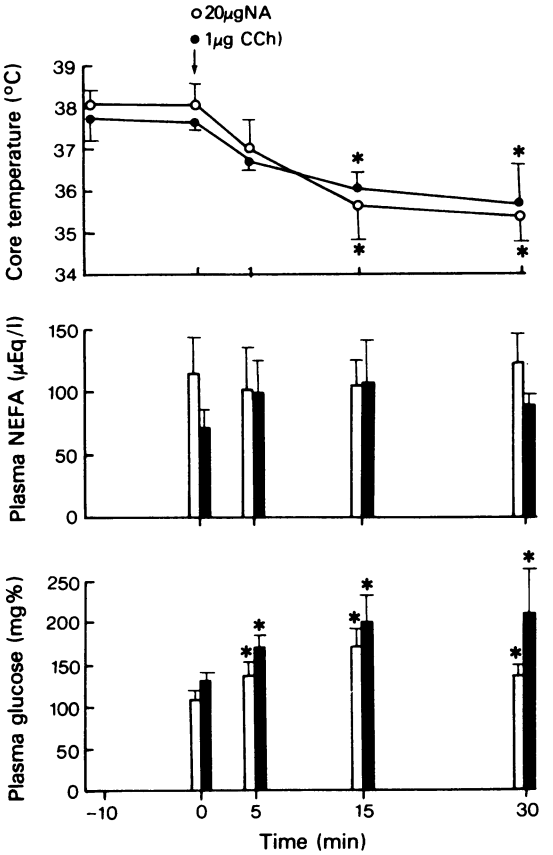

Figure 4 The changes (mean ± s.d., $n \geq 5$) in core temperature, plasma non-esterified fatty acid (NEFA) concentrations and plasma glucose concentrations measured 5, 15 and 30 min after injections of noradrenaline (NA, 20 μg, open circles or columns) and carbachol (CCh, 1 μg, closed circles or columns) into the anterior hypothalamus of rats at 23°C. * P values ranged from $P < 0.025$ to $P < 0.001$.

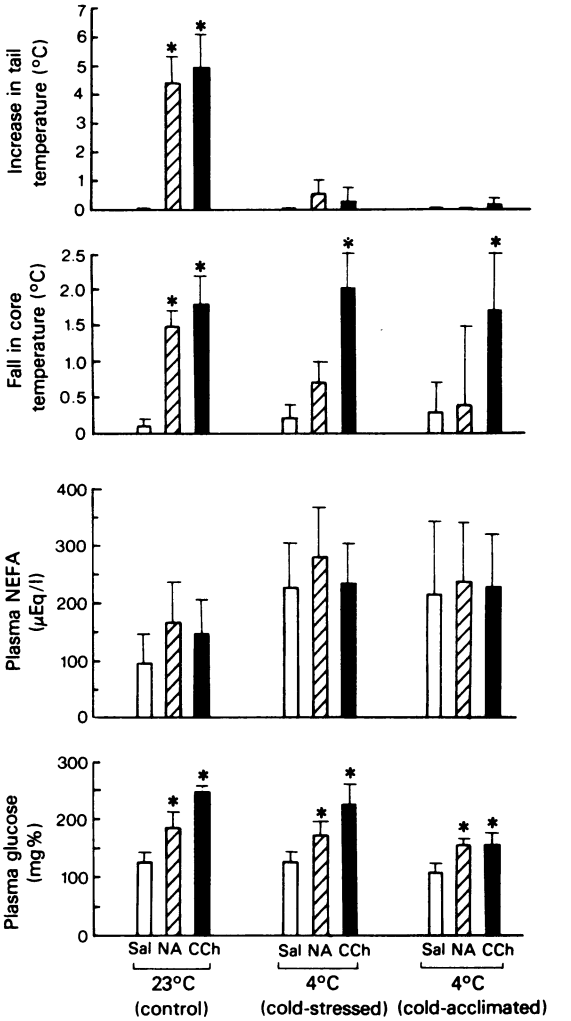

Figure 5 The changes (mean ± s.d., $n \geq 5$) in core temperature, tail temperature, plasma non-esterified fatty acid (NEFA) concentrations and plasma glucose concentrations evoked 15 min after injections of saline (Sal, open columns) noradrenaline (NA, 20 μg, hatched columns) and carbachol (CCh, 1 μg, closed columns) into the anterior hypothalamus of control, acutely cold-stressed and cold-acclimated rats. * P values ranged from $P < 0.01$ to $P < 0.001$.

at a thermoneutral ambient temperature. These temperature responses indicate that, at thermoneutrality, noradrenaline and carbachol increase heat loss, the heat being dissipated by vasodilatation of the tail blood vessels. In cold-stressed and cold-acclimated rats only carbachol evoked significant falls in core temperature and neither amine increased tail temperature. It has been suggested that there are thermal receptors in the tail skin which respond to changes in ambient temperature (Bazett, 1949). Therefore the absence of tail blood vessel dilatation in the cold after noradrenaline and carbachol could be explained by the thermal receptors acting via a local reflex to maintain vasoconstriction and prevent the vasodilatation expected after intrahypothalamic amine injections and observed at thermoneutrality. Since noradrenaline did not significantly lower core temperature in cold-exposed rats, it is likely that noradrenaline-induced hypothermia at thermoneutrality is mediated by increased non-evaporative heat loss, e.g. increased tail temperature, an effect which is not elicited in cold-stressed or cold-acclimated rats. An additional action on metabolism at thermoneutrality is unlikely

since noradrenaline elicited similar increases in plasma glucose levels of normal, cold-stressed and cold-acclimated rats but only evoked significant falls in core temperature at thermoneutrality. This reduced effect of noradrenaline in rats exposed to cold cannot easily be compared with the results of Schmidt (1963), who found that the decreases in rectal temperature after noradrenaline injections (10 μg in 10 μl) into unspecified sites in the cerebellum and cerebrum of 'grouped' rats were greater in cold-acclimated rats than in rats at a thermoneutral ambient temperature.

In cold-exposed rats, carbachol evoked hypothermia without increasing tail temperature, i.e. heat loss from vasodilatation of tail blood vessels. Assuming that the absence of increased heat loss from the tail reflects on absence from other sites (e.g. the scrotum and paws), carbachol must have decreased metabolic heat production since core temperature may be lowered only by increased heat loss or decreased heat production. This would probably effect a greater reduction in the core temperature of cold-exposed rats because of the larger core/ambient temperature gradient, an observation supported by the finding that carbachol-induced hypothermia was similar to that obtained in rats at thermoneutrality when increased heat loss contributed to the hypothermia. These results are consistent with a report of inhibition of non-shivering thermogenesis and hypothermia after injections of carbachol into the preoptic area of cold-exposed young guinea-pigs (Zeisberger & Bruck, 1971a).

Noradrenaline and carbachol evoked substantial increases in plasma glucose concentrations when injected into the anterior hypothalamus of normal, cold-stressed and cold-acclimated rats but the increases were not related to the degree of hypothermia. Furthermore, increases in plasma glucose concentrations could not be compared with changes in glucose turnover because the estimation of turnover from the specific activity of expired CO_2 is dependent on a constant plasma glucose concentration. The necessarily large 'dead space' of tubing required to remove blood samples within a short space of time precluded direct estimation of glucose turnover is conscious unrestrained rats; and the amine-induced increases in plasma glucose concentrations were abolished in rats anaesthetized with the following anaesthetics: chloral hydrate (30 to 40 mg/100 g, BDH), ketamine (Ketalar, 2 mg/100 g i.p. initially, then 0.1 mg 100 g^{-1} min^{-1}, Parke-Davis), and alphaxalone:alphadolone acetate (9 mg:3 mg/ml, Althesin, 0.1 ml/100 g initially, then 0.02 ml 100 g^{-1} min^{-1}, Glaxo) (Poole & Stephenson, unpublished observations). Therefore it was not possible to measure the effects of noradrenaline and carbachol on glucose turnover in anaesthetized animals.

In contrast to the marked increases in plasma glucose concentrations which were evoked by intrahypothalamic injections of noradrenaline and carbachol, changes in plasma NEFA were minimal. These minimal effects contrast with those evoked by injections of noradrenaline or an acetylcholine/physostigmine solution into the cerebral ventricles of sheep and oxen. While having little effect at thermoneutrality, noradrenaline significantly decreased plasma NEFA levels in cold-stressed oxen (Thompson & Clough, 1971) and sheep (Darling & Thompson, 1973). Intraventricular injections of an acetylcholine/physostigmine solution increased plasma NEFA levels of sheep at thermoneutrality (Darling & Thompson, 1973: Darling, Findlay & Thompson, 1974) but decreased plasma NEFA levels of cold-stressed sheep (Darling & Thompson, 1973). It is possible that noradrenaline and carbachol decreased oxidation of plasma NEFA without affecting mean plasma concentrations; unfortunately estimation of NEFA turnover was not possible in conscious unrestrained rats for the technical reasons described above, or in anaesthetized animals because control plasma NEFA concentrations were approximately doubled in rats anaesthetized with chloral hydrate, ketamine or alphaxalone/alphadolone acetate (Poole & Stephenson, unpublished observations).

Although amine-induced increases in plasma NEFA concentrations were not significant, plasma NEFA levels were elevated in both acutely cold-stressed and cold-acclimated rats. The elevation in cold-stressed rats is consistent with the existing literature (see review by Himms-Hagen, 1972) and emphasizes the importance of lipid as a substrate during cold-exposure. In contrast, the increased plasma NEFA concentrations in the rats which were cold-acclimated in the present study does not agree with the results of Masironi & Depocas (1961), who reported less than normal concentrations of NEFA in the plasma of cold-acclimated rats living in the cold (6°C). However, lipid is not the sole substrate used to support the cold-induced increase in metabolic rate: although plasma glucose concentrations were unaffected by cold-exposure, glucose oxidation rate was increased. This result accords with a report that the liver increases its output of glucose into the circulation during acute cold stress (Thompson, 1977). Consequently, plasma glucose concentrations need not necessarily increase: no significant increases were seen in the present study, when rats acclimated to 23°C were acutely exposed to 4°C, although Depocas & Masironi (1960) reported small increases in plasma glucose concentrations, when rats acclimated to 30°C were acutely exposed to 6°C. The similarity of plasma glucose concentrations of control and cold-acclimated rats in the present study confirms the results of Depocas & Masironi (1960) but conflicts with a report of decreased plasma glucose concentrations in cold-acclimated rats (Beck, Zaharko & Kalser, 1967).

A likely cause of the conflict is the substantially lower body weights of the rats cold-acclimated by Beck *et al.*, which weighed 56% of controls after 4 weeks cold exposure compared to 88% in the present study. This difference was maintained after 6 to 8 weeks cold exposure, when rats cold-acclimated by Beck *et al.* weighed 61% of controls compared to 89% in the study of Depocas & Masironi (1960).

There was no evidence in the present study for an accelerated Cori cycle, i.e. circulation of muscle lactate to the liver for glucose synthesis, in acutely cold-stressed or cold-acclimated rats. The data for acutely cold-stressed rats are consistent with the data obtained in acutely cold-stressed cattle, sheep and dogs (see review by Thompson, 1977). Minaire, Pernod, Jomain & Mottaz (1971) reported a large increase in [^{14}C]-lactate turnover and conversion to $^{14}CO_2$ in the bodies of dogs but no increase in circulating lactate, suggesting that increased lactate production and utilization were confined to muscles.

Since noradrenaline-induced hypothermia was almost abolished rather than enhanced when rats were cold-stressed, and carbachol-induced hypothermia was unaffected by cold stress, the neuronal model formulated by Bligh *et al.* (1971) for sheep, goats and rabbits is not applicable to rats. A species difference also exists between rats and guinea-pigs since intrahypothalamic injections of noradrenaline have been reported to activate non-shivering thermogenesis and increase core temperature in cold-stressed young guinea-pigs (Zeisberger & Bruck, 1971b). Finally, the similarity of the responses to noradrenaline and carbachol in acutely cold-stressed and cold-acclimated rats suggests similar stimulation of efferent pathways from the hypothalamus irrespective of whether rats are maintaining body temperature by shivering thermogenesis or by non-shivering thermogenesis.

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