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Changes in body core and skin temperature following intracerebroventricular injection of substances in the conscious rat: interpretation of data

During experiments to investigate the role of proposed transmitter substances in regulating body temperature in the conscious rat, it became apparent that many such substances injected intracerebroventricularly (i.c.) caused a fall in core temperature, accompanied by a rise in skin temperature. Accordingly, it was decided to extend the initial study to include a wider range of naturally occurring and synthetic substances, to see if any correlation could be established between the known receptor activity of these drugs and the response obtained.

A permanent cannula was implanted in the left lateral ventricle of male hooded rats by the method of Hayden, Johnson & Maickel (1966). All drugs were dissolved in 0·9% w/v sterile saline and injected in a volume of 10 μl/rat. Oesophageal and skin temperature (base of tail) were measured using probes connected to an electric thermometer. The temperature changes quoted relate to mean differences from the temperature immediately before injection.

Drugs that are adrenoceptor agonists, or mediate their effects at or through dopamine or 5-hydroxytryptamine (5-HT) receptors, caused a significant fall in core temperature ($P < 0{\cdot}05$) and a significant rise in skin temperature ($P < 0{\cdot}05$) following i.c. injection (Table 1). The peak responses of core temperatures usually occurred after 10 min with all drugs except oxymetazoline, ergotamine and apomorphine, for which the peak fall occurred after 25 min. Peak rises in skin temperature invariably occurred after 5 min, with the exception of the latter three substances to which, the peak response occurred after 15–25 min. Acetylcholine and vasopressin also caused hypothermia after i.c. injection which was preceded by a rise in skin temperature. Papaverine, salbutamol and isoprenaline did not modify core temperature following i.c. injection. However, salbutamol and papaverine, but not isoprenaline, lowered skin temperature which for salbutamol was a significant effect ($P < 0{\cdot}05$).

The fall in core temperature is secondary to the rise in skin temperature. The latter effect results from vasodilation of peripheral vessels and can only be due to inhibition of sympathetic tone. Thus peripheral vasodilation is the common mediating mechanism whereby loss of core heat is effected. Centrally however, several mechanisms could control such peripheral changes e.g. (i) Activation of thermoregulatory centres; (ii) activation of sympathoinhibitory centres; (iii) alterations in local cerebral blood flow.

Firstly, in accord with Feldberg & Myers (1964), the drugs could all be acting on neurons mediating thermoregulatory heat dissipating mechanisms. It is remarkable, if this is the case, that such a variety of drugs all affect body temperature in a qualitatively similar fashion. Folkow, Johansson & Oberg (1959), Lofving (1961) and Folkow, Langston & others (1964) have shown that electrical stimulation of the anterior hypothalamic region results in generalized peripheral sympathetic inhibition. It is possible, therefore, that the observed effects could simply arise following activation of

central sympathoinhibitory centres in the anterior hypothalamus which in turn would cause peripheral vasodilation and subsequent fall in core temperature. Intracerebroventricular injections of noradrenaline lower peripheral blood pressure (Smookler, Severs, & others, 1966; Share & Melville, 1963) and 5-HT applied by this route has a similar effect (Kaneko, McCubbin & Page, 1960). Activation of sympathoinhibitory centres seems to be the mechanism by which α-methyldopa and clonidine are thought to have their hypotensive effects (Henning & Rubenson, 1970; Struyker Boudier & Van Rossum, 1972).

The third interpretation concerns the influence on hypothalmic blood flow of the drugs injected into the brain. McCook, Peiss & Randall (1962) have suggested that arterial blood cools the hypothalamus and this proposition was supported by Hassler & McCook (1971), who found that occlusion of the blood flow to the hypothalamus raised hypothalamic temperature. As thermosensitive neurons are present in the anterior hypothalamus (Hellon, 1967) changes in hypothalamic temperature could activate thermoregulatory mechanisms. If blood flow to the hypothalamus was decreased by vasconstriction local rises in hypothalamic temperature could occur, activating heat dissipating mechanisms which in the rat involve vasodilation in the tail (Rand, Burton & Ing, 1965). An increase in blood flow through the hypothalamus as would occur during vasodilation, could possibly exert a qualitatively opposite

Table 1. *Effect of i.c. injections on body core and skin temperature in the conscious rat.* Mean responses are expressed as differences from the temperatures immediately before the injection ±s.e.; the results are the means of at least 6 determinations.

Drug	Dose μg/rat	Maximum mean fall °C of core temperature	Maximum mean rise °C in skin temperature
Noradrenaline	20	−1·2 ± 0·10	+4·5 ± 0·41
Oxymetazoline	10	−1·4 ± 0·23	+4·9 ± 1·04
Adrenaline	10	−0·4 ± 0·12	+2·6 ± 0·66
Ergotamine	5	−0·7 ± 0·21	+3·4 ± 0·34
Dopamine	50	−1·3 ± 0·23	+4·3 ± 0·13
Apomorphine	30	−1·0 ± 0·32	+3·9 ± 0·72
Amphetamine	100	−1·3 ± 0·31	+4·9 ± 0·42
5-HT	20	−1·3 ± 0·22	+5·0 ± 0·63
Norfenfluramine	100	−1·8 ± 0·14	+6·2 ± 1·12
Acetylcholine	100	−0·8 ± 0·14	+4·0 ± 0·75
Vasopressin	0·01 i.u.	−0·7 ± 0·24	+4·2 ± 0·65
Papaverine	50	Inactive	−0·8 ± 0·32
Salbutamol	100	Inactive	−1·4 ± 0·29
Isoprenaline	100	Inactive	Inactive
Saline		Inactive	Inactive

Although the response at only one dose level is shown, when activity occurred a dose-response relation was obtained for both core and skin temperature.

effect and some evidence for this is seen in the results obtained after i.c. injections of papaverine and salbutamol which caused a fall in skin temperature. Rosendorff & Cranston (1971) measured blood flow through the rabbit hypothalamus after i.c. injections of noradrenaline and 5-HT, and found that noradrenaline increased blood flow and caused a rise in body core temperature, while 5-HT decreased blood flow and caused a fall in core temperature. These findings are consistent with the propositions made above.

Without simultaneous measurements of central and peripheral blood flow, and body core and skin temperatures, it is not easily possible to identify the mechanisms by

which drugs injected i.c. mediate their effects on body temperature in the conscious rat. Certainly, the diversity of drugs producing a similar response pattern suggests that a single mechanism by which temperature changes occur is unlikely.

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A new method for the rapid determination of azovan blue leaked into the skin

There have been many methods for the determination of azovan blue leaked into the ventral skin after intravenous injection. These are all time consuming. Recently, an improved method was published (Harada, Takeuchi & others, 1971) but this required more than 24 h to complete the determination. We have developed a new method for the estimation of azovan blue which depends on the hydrolysis by lactic acid and hydrochloric acid of collagen composing the skin. The major advantages are that it is sensitive, reproducible with little degradation of the dye and convenient for the analysis of a large number of samples in a day.

Skin containing azovan blue is added to 3 ml of 28% lactic acid and boiled for 5 min. Then, 3 ml of 9 N HCl is added and the solution boiled for 5 min. After vigorous shaking the solution is cooled to room temperature (20°) and about 5 ml of CCl_4 is added with shaking. After centrifugation at 2000 rev/min for 5 min, the solution separates in two phases. The lower phase is removed and 5 ml of acetone is added to the tube. The supernatant obtained after shaking and then centrifugation at 3000 rev/min for 10 min is used for the colorimetric determination of the dye at 615 nm. The colour is stable when measured within 2 h.

A standard curve can be obtained by adding amounts of dye in 0·05 ml of 0·85% NaCl solution to a test tube to which a piece of the intact skin is added. The standard curve is linear over the range 25 to 125 μg of azovan blue.