Scatchard, G. (1949) Am. N.Y. Acad. Sci. 51: 660–672
Schulz, R., Wuster, M., Herz, A. (1979) Naunyn Schmiedeberg's Arch. Pharmacol. 306: 93–96
Shaw, J. S., Miller, L., Turnbull, M. J., Gormley, J. J., Morley, J. S. (1982) Life Sci. 31: 1259–1262
Wood, P. L., Charleson, S. E., Lane, D., Hudgin, R. L. (1981) Neuropharmacology 20: 1215–1220
Zukin, R. S., Sugarman, J. R., Fitz-Syage, M. L., Gardner, E. L., Zukin, S. R., Gintzler, A. R. (1982) Brain Res. 245: 285–292

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The development of a relative hyperthermia during continuous clonidine infusion in the rat

C. MACCARRONE*, S. J. LEWIS, B. JARROTT, *University of Melbourne, Department of Medicine, Clinical Pharmacology & Therapeutics Unit, Austin Hospital, Heidelberg, Vic., 3084 Australia*

In rats the continuous infusion of clonidine (10 μg kg^{-1} h^{-1}) produces a relative hyperthermia of approximately 0·6 °C during the light phases but not the dark phases of a 10 day infusion period. It appears that the relative hyperthermia results from a clonidine infusion-induced attenuation of the fall in body temperature which normally occurs at the onset of the light phases.

In rats, the acute systemic injection of clonidine produces a dose-dependent hypothermia (Ozawa et al 1977; Bugajski et al 1980). This response most probably arises from the direct stimulation of α_2-adrenoceptors (Zacny 1982) within the thermoregulatory control centres of the hypothalamus and brainstem (Tsoucaris-Kupfer & Schmitt 1972a, b). In addition, it has been suggested that this fall in body temperature results from a decrease in metabolic heat production as well as increases in evaporative heat loss and cutaneous vasodilatation (Lin et al 1981). At present, little is known concerning the thermoregulatory effects of longer term clonidine administration. Lewis et al (1981) examined the effects of twice daily intraperitoneal injections of clonidine (50 μg kg^{-1}) for 7 days and reported that a hypothermia of 3–4 h duration followed each injection. However, this drug has a relatively short elimination half-life (1 h) in the rat (Conway & Jarrott 1980) and so this injection regimen cannot be regarded as a suitable form of chronic administration. However, with the availability of implantable osmotic minipumps which can infuse drugs continuously at a constant rate for up to 14 days, this problem can be circumvented.

We now report that rats receiving a subcutaneous infusion of clonidine (10 μg kg^{-1} h^{-1} via osmotic minipumps), at a rate which is essentially devoid of any initial changes in body temperature, produced a relative hyperthermia by day 2 of a 10 day infusion period. Moreover, the elevation in body temperature is restricted to the light phases (08.00–20.00 h) rather than the dark phases (20.00–08.00 h) of the infusion period suggesting that these effects may result from an interaction with circadian control mechanisms.

* Correspondence.

Methods

Female Wistar-Kyoto rats weighing between 205–225 g were used. Animals with similar body temperatures (within a range of 0·3 °C) were deliberately selected for the experiments. Immediately before and during the experiments the animals were kept in individual cages with food and water freely available. The room was maintained at a temperature of 21 ± 1 °C and had a 12 h light (08.00–20.00 h)—dark cycle. In order to obtain constant tissue and fluid concentrations of clonidine, this drug was continuously infused at a rate of 10 μg (base) kg^{-1} h^{-1} in 8 rats via subcutaneously implanted osmotic minipumps (Model 2002; ALZA Corporation, California, USA). This infusion rate was selected on the basis that it does not initially lower the body temperature in the rats. Higher infusion rates of 50–100 μg kg^{-1} h^{-1} do produce hypothermia (Maccarrone, unpublished observation). The minipumps were implanted at 20.00 h (beginning of day 1) under light halothane anaesthesia whilst sham operated rats (n = 8) served as controls. The body temperatures of these unrestrained rats were measured once every hour during days 1, 2, 6 and 10 of the infusion by means of a thermistor probe inserted 6 cm into the rectum and recorded with the aid of a tele-thermometer (Model 423, Yellow Springs Instrument Co., USA). Preliminary studies have demonstrated that the infusion of 0·9% NaCl (saline) from these minipumps does not affect a variety of physiological parameters including body temperature (Jarrott & Lewis, in preparation).

The values represented here are the mean ± s.e.m. Statistical analysis of differences between means were performed by one way or two way analysis of variance (ANOVA). The analysis is by one way ANOVA unless stated otherwise.

Results

The body temperatures of the control rats displayed a circadian rhythm with the values being consistently higher during the dark phases compared with the light phases on each day of the experiment. For example, on day 6, the body temperature was on average 0·9 °C higher during the dark phase than the following light phase (Table 1). These changes were clearly light/dark

Table 1. The body temperature of control rats and those receiving clonidine infusion (10μg kg^{-1} h^{-1}) on day 6 of the infusion. The clonidine-containing minipumps were implanted at 20.00 h on day 1. Each value represents the combined mean ± s.e.m. of the 12 mean hourly temperatures (n = 8 animals per group) for the dark (20.00–08.00 h) and light (08.00–20.00 h) phases. Statistical analysis was performed on the individual means by 2 way ANOVA (testing for the effects of drug versus time). Significant differences between means were taken as $P < 0.05$.

Group	Body temperature (°C) Dark phase	Light phase
Control	38·3 ± 0·09	37·4 ± 0·11[a]
Clonidine	38·5 ± 0·02[c]	38·0 ± 0·04[b,d]

[a] $P < 0.005$; 1,11 d.f. F = 75·4 light phase control versus dark phase control.
[b] $P < 0.005$; 1,11 d.f. F = 65·8 light phase clonidine versus dark phase clonidine.
[c] $P > 0.25$; 1,11 d.f. F = 1·9 dark phase clonidine versus dark phase control.
[d] $P < 0.005$; 1,11 d.f. F = 32·9 light phase clonidine versus light phase control.

phase dependent with the body temperatures falling to the lower plateau within 2 h of commencement of the light phase. For example, on day 6 at 08.00 h (commencement of light phase) versus 10.00 h (2 h into light phase) the body temperatures were respectively 38·5 ± 0·09 °C versus 37·2 ± 0·09 °C (1, 14 d.f. F = 104·9; $P < 0.001$ see Fig. 1). Conversely, the body temperature of the rats rose to higher plateau values within 2 h of commencement of the dark phase. For example, on day 6 at 20.00 h (commencement of dark phase) versus 22.00 h (2 h into dark phase) the body temperatures were respectively 37·4 ± 0·13 °C versus 38·5 ± 0·15 °C (1,14 d.f. F = 28·2; $P < 0.001$ see Fig. 1).

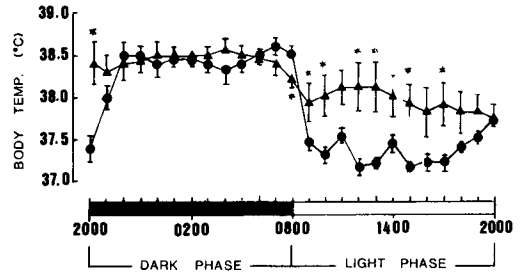

FIG. 1. The body temperature of sham operated rats (●) and those receiving clonidine infusion (10 μg kg^{-1} h^{-1},. ▲) over the dark (20.00–08.00 h) and light (08.00–20.00 h) phases of day 6. Each point represents the mean ± s.e.m. from 8 rats. Asterisks indicate significant differences ($P < 0.05$, one way ANOVA) between the mean values of the sham and clonidine infusion groups.

During the first 36 h, the body temperatures of the rats receiving the clonidine infusion were not different to those of controls ($P > 0.05$ for all comparisons). However, a significant clonidine-induced elevation in body temeprature was observed at 09.00 h on day 2 of infusion (i.e. after 37 h; clonidine infusion versus control, 38·4 ± 0·16 °C versus 37·9 ± 0·11 °C; $P < 0.03$). This hyperthermia was maintained throughout the light phase (38·2 ± 0·04 °C versus 37·6 ± 0·08 °C; 1,11 d.f. F = 65·8; $P < 0.005$, 2 way ANOVA) with the maximal effects occurring between 14.00–19.00 h (e.g. at 18.00 h, 38·3 ± 0·13 °C versus 37·4 ± 0·08 °C, $P < 0.001$). The body temperatures of the control and treated groups were not different during the dark phases of day 6 (Table 1) and day 10. Except for day 1, the clonidine-induced elevations in body temperature were observed during all the light phases in which body temperature was monitored (i.e. Days 2, 6 and 10, see Table 1 and Fig. 1 for day 6). It appears that this relative hyperthermia results from a clonidine infusion-induced attenuation of the fall in body temperature which normally occurs immediately after the onset of the light phase. As described above, this fall is approximately 0·9 °C for the control group, but only 0·3–0·5 °C for the clonidine group (from day 2 onwards).

Discussion

These results demonstrate that the continuous infusion of clonidine at a rate which is devoid of initial effects on body temperature produces a relative hyperthermia in the rat by an apparent modulation of the circadian rhythm of body temperature. The precise mechanisms underlying the clonidine-induced hyperthermia remain to be established. However, this effect may result from the clonidine-induced decrease in effectiveness of central hypothermic systems which are recruited to maintain body temperature at lower levels during the day (Bligh 1979). Such an effect may possibly result from clonidine producing biochemical changes within these systems, for example a subsensitivity of α-adrenoceptors. Indeed, Engberg et al (1982) demonstrated that chronic clonidine treatment results in a reduced sensitivity of α_2-adrenoceptors on noradrenergic neurons of the locus coeruleus. In addition, drugs which interact with aminergic systems can influence the circadian cycle of α-adrenoceptor densities (Wirz-Justice et al 1980). Consequently, if the clonidine infusion produces an effective down regulation of α-adrenoceptors, then the relative hyperthermic effects of the infusion may result from the reduced activity of endogenous hypothermic neurotransmitter pathways which subserve stimulation of this receptor type. However, the elevations in body temperature during the light phase may also result from other mechanisms such as the active recruitment of hyperthermic pathways.

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REFERENCES

Bligh, J. (1979) Neuroscience 4: 1213–1236

Bugajski, J., Zacny E., Zdebska, A. (1980) Neuropharmacology 19: 9–15

Conway, E. L., Jarrott, B. (1980) Br. J. Pharmacol 71: 473–478

Engberg, G., Elam, M., Svensson, T. H. (1982) Life Sci. 30: 235–243

Lewis, S. J., Fennessy, M. R., Taylor, D. A. (1981) Clin. Exp. Pharmacol. Physiol. 8; 489–495

Lin, M. T., Chandra, A., Ko, W. C., Chen, Y. M. (1981) Neuropharmacology 20: 15–21

Ozawa, H., Chen, C. S., Watanabe, H., Uematsu, T. (1977) Japan J. Pharmacol. 27: 47–54

Tsoucaris-Kupfer, D., Schmitt, H. (1972a) C.R. Acad. Sci. 274: 607–610

Tsoucaris-Kupfer, D., Schmitt H. (1972b) Neuropharmacology 11: 625–635

Wirz-Justice, A., Kafka, M. S., Naber, D., Wehr, T. A. (1980) Life Sci. 27: 341–347

Zacny, E. (1982) J. Pharm. Pharmacol. 34: 455–456

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Murine lymphocytes lack clearly defined receptors for muscarinic and dopaminergic ligands

DAVID E. WAZER*, JOHN ROTROSEN, *Psychiatry Service, New York Veterans Administration Medical Center and Department of Psychiatry, New York University School of Medicine, New York, USA*

[^{3}H]Quinuclidinyl benzilate and [^{3}H]spiperone binding to murine lymphocytes is displaceable but differs from binding to brain receptor sites for these ligands: (1) binding to intact lymphocyte preparations was not saturable; (2) disruption of intact lymphocytes was associated with a marked loss of displaceable ligand binding; (3) drugs differentially displace these ligands in lymphocytes compared to brain. Displaceable binding was increased following incubation of lymphocytes under phospholipid methylating conditions; however, marked effects on cell viability and cell recovery make it difficult to interpret these binding changes. If dopaminergic and cholinergic receptors do exist on lymphocytes, their binding characteristics are profoundly different from comparable cns receptors.

We have noted papers in recent years describing dopaminergic and muscarinic binding sites on mammalian lymphocytes. Uzan et al (1981) characterized [^{3}H]spiperone binding in murine lymphocytes with apparent B-cell specificity. Binding was stereospecific, saturable (Kd ~ 5 nM) and showed time course dissociation characteristics consistent with a 'classic' pharmacological receptor (Uzan et al 1981). Further, those authors reported data (Le Fur et al 1981) suggesting a coupling between the 'dopaminergic' receptors and phospholipid methylation.

Several authors (Zalcman et al 1981; Strom et al 1981; Gordon et al 1978) describe muscarinic cholinergic binding sites on human and murine lymphocytes. These sites are T-cell specific and saturable (Kd 10-100 nM) and are retained in homogenized tissue preparations (Bidart et al 1983).

Maloteaux et al (1982) question the existence of both dopamine and muscarinic receptors on human lymphocytes. Those authors report that the potencies of various drugs in displacing labelled ligands differ markedly between human lymphocyte and rat striatal preparations. They hypothesize that labelled ligand is entrapped within cellular compartments of lymphocytes and may be mistakenly interpreted as 'specific binding'.

Because of our interest in both of these receptors and the interactive role of phospholipids on receptor function, we have studied [^{3}H]spiperone ([^{3}H]SPD) and [^{3}H]quinuclidinyl benzilate ([^{3}H]QNB) binding in murine lymphocytes. Based on our data to date, we share some of the reservations of Maloteaux et al and similarly question whether the binding data clearly support the existence of receptors. We wish to briefly report our observations.

Methods and results

Spleens were collected from male Swiss-albino mice (20–30 g). All dilutions were performed with Hank's balanced salt solution and lymphocytes isolated by means of a Ficoll gradient procedure (cf. Uzan et al 1981). Cells were washed twice with Hank's solution and counted. Viability of lymphocytes (erythrocin B exclusion) exceeded 97%.

In-vitro 'binding' assays were performed using 1–3 × 10^6 cells/tube and either [^{3}H]SPD (specific activity—21·0 Ci mmol^{-1}) or [^{3}H]QNB (specific activity—30·2 Ci mmol^{-1}) in a total volume of 1·0 ml. Incubations were for 1 h at 37 °C for [^{3}H]SPD binding and at 25 °C for [^{3}H]QNB binding. 'Specific binding' was defined in the presence or absence of 10^{-5} M haloperidol or 10^{-4} M atropine in the SPD and QNB binding respectively.

In order to address the 'binding'/'entrapment' issue we examined both intact and particulate tissue preparations. Particulate tissue preparations were prepared by Polytron action (setting 5; 2 × 30 s bursts).

67% of total SPD binding and 33% of total QNB binding was found to be displaceable. We were unable to achieve saturation in intact lymphocytes for either [^{3}H]SPD (up to 5000 nM) or [^{3}H]QNB (up to 250 nM). In

* Correspondence.