Effects of GABA agonists and antagonists on temperature-sensitive neurones in the rat hypothalamus

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1. Extracellular recordings were obtained from 94 warm-sensitive, 6 cold-sensitive and 117 temperature-insensitive neurones in slices of the hypothalamic medial preoptic area of rats, to determine the effect of the $GABA_A$ agonist muscimol, the $GABA_A$ antagonist bicuculline, the $GABA_B$ agonist baclofen and the $GABA_B$ antagonist phaclofen on tonic activity and temperature sensitivity.

2. Muscimol and baclofen dose-dependently inhibited the tonic activity of 69% (36/52) and 97% (36/37) of the hypothalamic neurones, respectively, regardless of their type of thermosensitivity. In contrast, the $GABA_A$ antagonist bicuculline increased the tonic activity of the majority of neurones (58/83), while the $GABA_B$ antagonist phaclofen increased neuronal activity only in the high dose of 100 μM.

3. The temperature sensitivity of hypothalamic neurones was only changed by ligands of $GABA_B$ receptors, and this effect was restricted to warm-sensitive neurones. The temperature coefficient (TC) was significantly increased by the $GABA_B$ agonist baclofen ($\Delta TC = 0{\cdot}69 \pm 0{\cdot}11$ imp s^{-1} $°C^{-1}$, $P < 0{\cdot}01$, $n = 18$). In contrast, the $GABA_B$ antagonist phaclofen (10 μM) decreased the temperature sensitivity ($\Delta TC = -0{\cdot}67 \pm 0{\cdot}09$ imp s^{-1} $°C^{-1}$, $P < 0{\cdot}01$, $n = 10$) in doses which did not affect tonic activity.

4. The increase in temperature sensitivity due to the $GABA_B$ agonist baclofen was significantly enhanced by co-perfusion of the $GABA_A$ antagonist bicuculline, indicating an interaction of $GABA_A$ and $GABA_B$ receptor-mediated mechanisms with regard to neuronal thermosensitivity.

5. The results suggest that neurones in the medial preoptic area are subject to GABA-mediated tonic inhibition resulting in modulation of tonic activity and temperature sensitivity of warm-sensitive neurones possibly involved in the control of body temperature. The data support the hypothesis that the hypo- or hyperthermic action of an endogenous substance is related to its effect on the thermosensitivity rather than on tonic activity of hypothalamic neurones.

The preoptic area of the anterior hypothalamus (PO/AH) contains neurones sensitive to changes in the local temperature which may also respond to stimulation of temperature receptors located at the body surface or the body core temperature (Boulant & Dean, 1986; Simon, Pierau & Taylor, 1986; Hori, 1991). The efferent signal of these neurones triggers effector responses to sustain body temperature within the normal range (Boulant, 1980). Central transformation of the afferent information into thermoregulatory efferent signals involves different transmitter substances and modulators. One neurotransmitter believed to take part in the neuronal control of body temperature is γ-aminobutyric acid (GABA) (Bligh, 1981). GABA has been demonstrated in relatively high concentrations in various hypothalamic nuclei, being highest in preoptic and anterior hypothalamic areas, and appears to

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be mainly associated with intrinsic hypothalamic neurones (Ottersen & Storm-Mathisen, 1984). It has been suggested that short axonal GABAergic neurones may form local networks modulating afferent temperature signals within the hypothalamus (Blatteis, 1981).

This hypothesis conforms to the observations that non-anaesthetized rats treated centrally (intracerebroventricular, intrahypothalamic) or systemically (intraperitoneal, intravenous) with GABA or GABA agonists develop *hypothermia*, whereas administration of GABA antagonists results in *hyperthermia* (Clark & Lipton, 1985; Serrano, Minano, Sancibrian & Duran, 1985; Yakimova & Ovtcharov, 1989). The mechanism by which GABA exerts its influence on thermoregulatory processes is unknown. A number of endogenous substances such as bombesin, prostaglandin E_2 (PGE_2) and thyrotropin-releasing hormone (TRH), which change body temperature by central and systemic application, change either the tonic activity or the temperature coefficient (TC) or both of warm-sensitive and sometimes temperature-insensitive hypothalamic neurones (Pierau, Schenda, Konrad & Sann, 1994). Thus it appears reasonable to assume that the hypothermia induced by GABA might also be due to modulation of the hypothalamic temperature-sensitive network. In this study we have investigated the action of GABA agonists and antagonists upon tonic activity and temperature sensitivity of neurones in the preoptic area of the anterior hypothalamus using an *in vitro* slice preparation. The aim of this study was to characterize the cellular mechanisms by which stimulation or blockade of $GABA_A$ or $GABA_B$ receptors of neurones of the medial preoptic area could result in an altered body temperature.

Since GABA effects are known to be produced through two types of receptors, $GABA_A$ and $GABA_B$, we have used agonists and antagonists against both types of receptors. GABA binding to the classical $GABA_A$ receptor directly opens Cl^--selective channels; this effect can be blocked by bicuculline. Activation of $GABA_B$ receptors by GABA or baclofen is mediated by a G protein and causes a decrease in Ca^{2+} conductance (Wu & Saggau, 1995) or an activation of a potential- or Ca^{2+}-dependent K^+ conductance (Bowery, 1993), effects that can be blocked by phaclofen.

The data indicate that the activity of the majority of warm-sensitive and temperature-insensitive PO/AH neurones is under tonic inhibitory GABAergic control mediated by $GABA_A$ and $GABA_B$ receptors. In contrast, the temperature sensitivity appears to be only affected in warm-sensitive neurones and mediated through $GABA_B$ receptors. These results are in agreement with the notion that changes in the temperature sensitivity of hypothalamic neurones are part of the mechanism involved in the hypothermia induced by GABA.

METHODS

Male Wistar rats, of 200–250 g body weight, were decapitated, and brains were promptly removed. Brain slices (400 μm) from the medial preoptic area were prepared and stored as previously described (Schmid & Pierau, 1993). In the recording chamber (described by Schmid & Pierau, 1993) the slices were continuously perfused with oxygenated (95% O_2 and 5% CO_2) artificial cerebrospinal fluid (ACSF) at 2·5 ml min^{-1}. The ACSF contained (mM): 124 NaCl, 5 KCl, 1·2 NaH_2PO_4, 1·3 $MgSO_4$, 26 $NaHCO_3$, 1·2 $CaCl_2$ and 10 glucose (Sigma).

Extracellular recordings were made with glass-covered platinum–iridium electrodes from neurones located in the PO/AH region, maximally 1·5 mm lateral to the third ventricle and 1 mm caudal to the anterior commissure mainly in the medial preoptic area. The temperature of the tissue slice was kept constant at 38 °C during the search for spontaneously active neurones. Sinusoidal temperature changes within the range of 35–41 °C were performed with the aid of a Peltier thermoassembly (rate 0·02 °C s^{-1}) starting from 38 °C (for further details see Schmid & Pierau, 1993). The temperature was measured with a fine copper–constantan thermocouple (0·5 mm diameter) close to the slice. Action potentials were recorded by conventional electrophysiological equipment and processed together with the temperature on a personal computer using a 1401 interface (CED (Cambridge Electronic Design)) and CED software spike 2, and a digital tape recorder (DAT, DTR 1800, Biologic, France).

Before application of the test substances, the temperature sensitivity of a given neurone was determined using two or three periodic temperature stimuli at intervals of 5 min. Only neurones with reproducible temperature responses were used for further examination. Superfusion of test substances was started not before 5 min after the last control temperature stimulus; GABA agonists were applied for 5 min and GABA antagonists for 10 min before the next temperature stimulus was performed. Superfusion returned to ACSF 3 min after this stimulus was completed and a further temperature stimulus was given after a delay of at least 10 min. A number of additional temperature stimuli were applied in anticipation of complete recovery. Only one neurone per slice was tested.

The following substances were tested: muscimol ($GABA_A$ agonist, 0·1–10 μM), bicuculline methiodide ($GABA_A$ antagonist, 5 and 10 μM), baclofen ($GABA_B$ agonist, 0·1–10 μM) and phaclofen ($GABA_B$ antagonist, 10 and 100 μM). In order to evaluate the specificity and duration of the effect of the antagonists, muscimol and baclofen (0·1 ml of 0·1 μM and 1 μM) were added as bolus to the perfusion of the appropriate antagonist.

Bicuculline methiodide (Sigma, 5 and 10 μM); muscimol (Sigma, 0·1, 1, and 10 μM); baclofen (Sigma, 0·1, 1 and 10 μM) and phaclofen (RBI, 10 and 100 μM), previously prepared as stock solutions, were diluted in ACSF just before application.

The temperature sensitivity of a neurone was calculated by a program relating the discharge rate of the neurone (bin width, 5 s) to the respective temperature, and fitting either one linear or two piecewise regression lines through the data (Schmid & Pierau, 1993). The slope of the steepest regression line covering at least 2 °C was regarded as the TC of the unit. Temperature-sensitive neurones are defined by a TC $\geqslant$ 0·8 imp s^{-1} $°C^{-1}$ for warm

Table 1. General characteristics of neurones in the medial preoptic area of rats

Neurone type	Mean firing rate (imp s^{-1})	Mean temperature coefficient (imp s^{-1} $°C^{-1}$)
Warm-sensitive ($n = 94$)	8·31 ± 1·57 (0·52–23·8)	1·58 ± 0·21 (0·81–3·85)
Cold-sensitive ($n = 6$)	3·99 ± 3·10 (1·42–13·8)	−1·02 ± 0·29 (−0·66 – −2·34)
Temperature-insensitive ($n = 117$)	4·14 ± 0·57 (0·13–12·0)	0·31 ± 0·07 (−0·46 – +0·77)

Values are mean ± S.E.M. Numbers in parentheses indicate the range.

Table 2. Latency of the maximal decrease and return of spontaneous activity after application of GABA agonists

Agonists	Dose (μM)	Latency of maximal effect (min)	Latency of return of activity (min)
Muscimol	0·1 (29)	6·3 ± 1·8	4·6 ± 1·3
	1·0 (23)	3·4 ± 1·2	7·5 ± 1·6
	10·0 (6)	1·8 ± 0·9	23·5 ± 2·1
Baclofen	0·1 (26)	3·0 ± 0·7	3·5 ± 0·6
	1·0 (19)	2·5 ± 0·4	3·8 ± 0·5
	10·0 (6)	2·1 ± 0·8	3·9 ± 1·2

Values are mean ± S.E.M. Muscimol, $GABA_A$ agonist; baclofen, $GABA_B$ agonist. Numbers in parentheses indicate number of neurones.

sensitivity and TC $\leqslant -0·6$ imp s^{-1} $°C^{-1}$ for cold sensitivity; all other neurones are by this definition temperature insensitive. Changes in neuronal tonic activity (firing rate) were calculated with the aid of the same computer program, providing information on the mean value of firing rate for the duration of 1 min, recorded just prior to and for the 2 min following each temperature stimulus. All data are presented as means ± S.E.M. For statistical evaluation Student's paired or independent t test was used.

RESULTS

A total of 217 hypothalamic neurones were used in this study. A similar number of warm-sensitive (94) and temperature-insensitive neurones (117) were selected to compare the effect of GABAergic substances in both populations; six cold-sensitive neurones were also tested. The mean firing rate of warm-sensitive neurones (8·31 ± 1·57 imp s^{-1}) was significantly higher ($P < 0·01$) than for the other types of neurones (Table 1).

An example of an original recording of a warm-sensitive hypothalamic neurone is given in Fig. 1, demonstrating an increasing activity with increasing temperatures. The firing was usually quite regular in most warm-sensitive neurones, but about 20% of the neurones demonstrated a bursting type of activity, which was more pronounced over a hyperthermic temperature range.

Effects of substances acting upon $GABA_A$ receptors

Spontaneous activity. Muscimol, an agonist of $GABA_A$ receptors, was used in fifty-two neurones in concentrations of 0·1, 1 and 10 μM. It reduced the spontaneous activity of 69% (36/52) of both warm-sensitive and temperature-insensitive PO/AH neurones in a dose-dependent manner (Figs 2, 5 and 6). The latency of the peak inhibition induced by muscimol superfusion was shortened with increasing dose (Table 2). After application of 0·1 μM muscimol the firing rate was reduced by 20–30% and the activity was

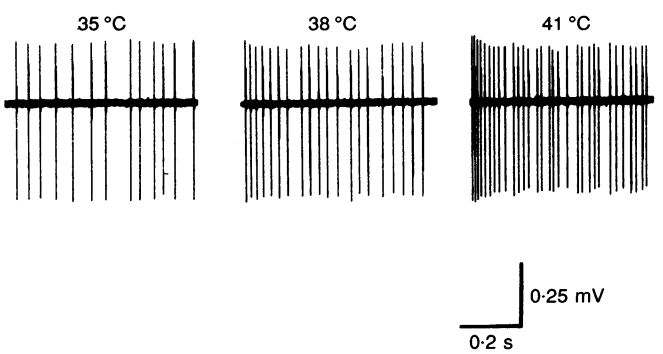

Figure 1. Effect of different temperatures on the tonic frequency of a rat warm-sensitive PO/AH neurone

Original extracellular recordings of a warm-sensitive PO/AH neurone at different slice temperatures. The signal-to-noise ratio was extremely good using glass-insulated platinium–iridium electrodes.

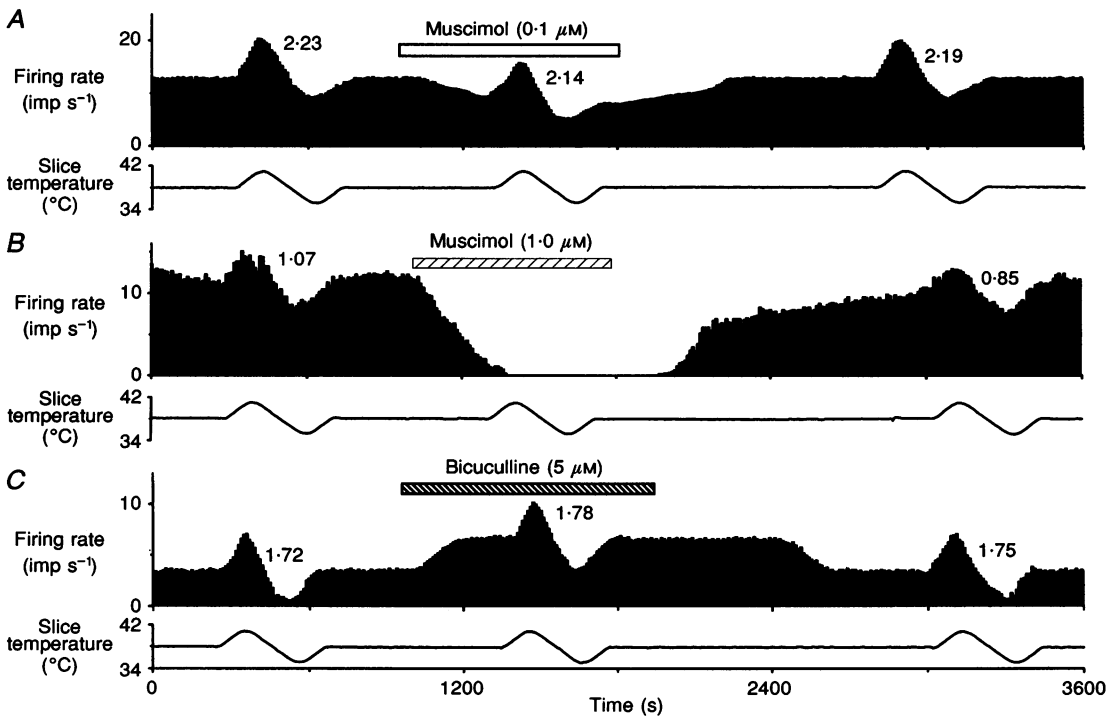

Figure 2. Effect of a $GABA_A$ agonist and antagonist on tonic activity and temperature response of warm-sensitive hypothalamic neurones

Original recordings of firing rate and slice temperature from 3 different PO/AH warm-sensitive neurones. *A*, superfusion with the $GABA_A$ agonist muscimol (0·1 μM) decreased firing rate but did not change the temperature sensitivity of the warm-sensitive neurone. *B*, superfusion with muscimol (1 μM) completely inhibited spontaneous discharges; the temperature stimulus was ineffective. *C*, superfusion with the $GABA_A$ antagonist bicuculline (5 μM) increased firing rate but did not change temperature sensitivity. The TC is indicated at the response to a temperature change.

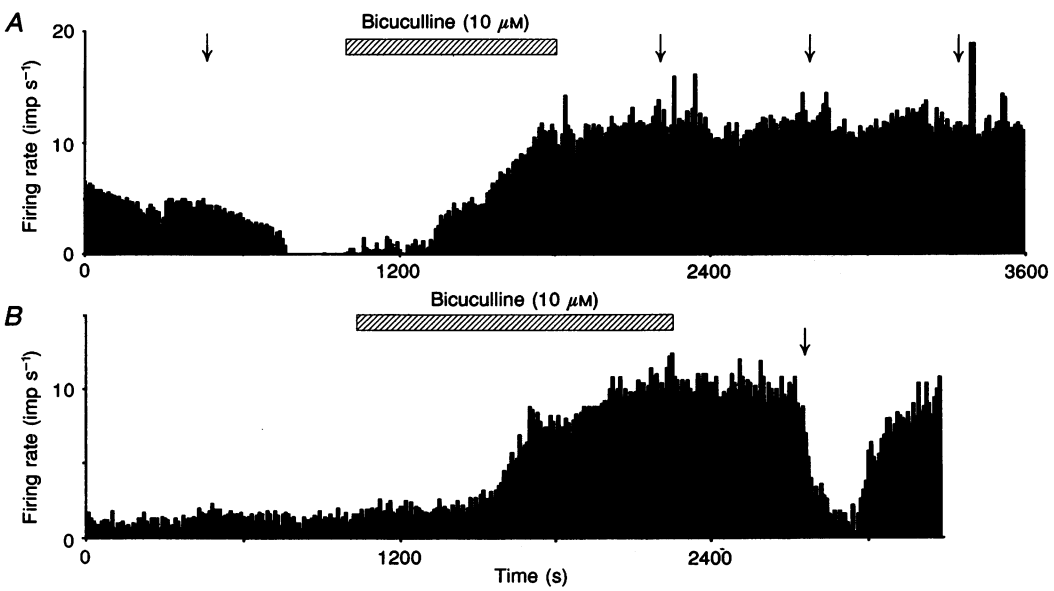

Figure 3. Specificity of the effect of a $GABA_A$ agonist and antagonist on spontaneous discharge

Original recordings of firing rate from 2 temperature-insensitive neurones of the medial preoptic area. *A*, the $GABA_A$ agonist muscimol (arrows, 0·1 ml of 1 μM, bolus injection) completely blocked neuronal activity. Superfusion with the $GABA_A$ antagonist bicuculline (10 μM) increased firing rate and cancelled the muscimol-induced inhibition for more than 30 min. *B*, superfusion with bicuculline (10 μM) increased firing rate but failed to block the inhibition induced by the $GABA_B$ agonist baclofen (arrow, 0·1 ml of 1 μM, bolus injection).

completely restored after 4·6 ± 1·3 min (Figs 2, 5 and 6, and Table 2). At high doses the activity of most (1 μM) or all (10 μM) neurones was reduced to zero (Fig. 2*B*), but complete recovery was not seen after prolonged washing periods (30–60 min, Fig. 6).

The $GABA_A$ antagonist bicuculline (5 and 10 μM) had the opposite effect on spontaneous activity of both types of hypothalamic neurones. It increased the firing rate of 70% of the neurones (58/83) in a dose-dependent manner (5 μM: by 1·86 ± 0·98 imp s^{-1}; $n = 22$; 10 μM: by 3·02 ± 1·28 imp s^{-1}; $n = 36$). This indicates that even in the slice preparation hypothalamic neurones are permanently affected by inhibitory GABAergic synapses. The increase in firing rate was most pronounced in neurones demonstrating low spontaneous activity (Figs 2*C* and 3*B*) and was accompanied by bursting discharges in 57% of the neurones. Additional small spikes were recruited during bicuculline application in about one-third of the recordings. These small action potentials were easy to identify and disappeared with the abolition of drug action. They most probably represent activity of previously silent neurones or axons in close proximity to the recorded neurone. The effect of bicuculline was quite persistent and outlasted the drug perfusion by 15–20 min (5 μM) and 30–40 min (10 μM; see Fig. 3).

Superfusion with bicuculline completely restored the neuronal activity inhibited by previous application of muscimol and totally prevented the inhibitory action of muscimol applied subsequently to bicuculline (Fig. 3*A*). This antagonistic action of bicuculline was via a specific $GABA_A$ mechanism, since bicuculline did not antagonize the inhibitory action of the $GABA_B$ agonist baclofen (Fig. 3*B*). An additional indication for the specificity of the $GABA_A$ agonists and antagonists was the observation that in the five neurones in which bicuculline was applied subsequently to muscimol (after the neurone had completely recovered from the muscimol effect) activation of tonic activity was only observed in those three neurones which had previously responded to muscimol.

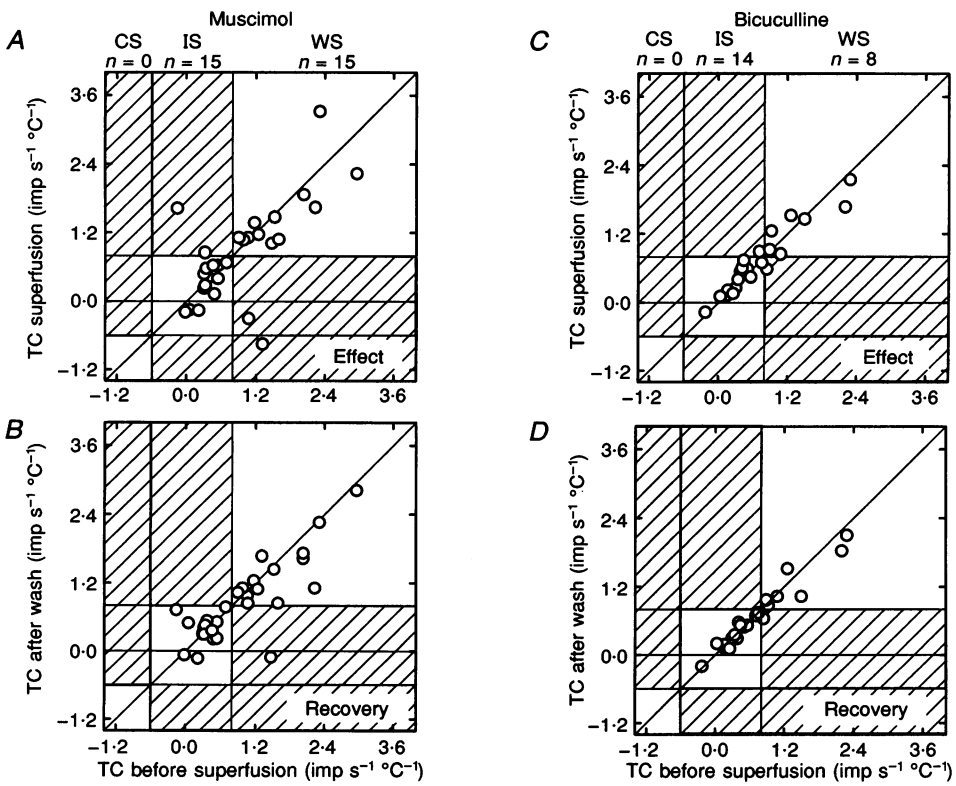

Figure 4. Effects of the $GABA_A$ agonist muscimol and antagonist bicuculline on temperature sensitivity of hypothalamic warm-sensitive neurones

To illustrate the TC changes of individual neurones the TC during application (effect) of muscimol (*A*) and bicuculline (*C*) and after washing (*B* and *D*, recovery) is plotted as a function of the TC during the control period. The distance of a circle from the line of identity indicates the degree of change. Circles in hatched areas represent neurones in which the TC changes were large enough to transform this neurone into another category. Whilst muscimol transformed 2 temperature-insensitive neurones into warm-sensitive ones and 2 warm-sensitive neurones into temperature-insensitive or cold-sensitive neurones, bicuculline did not produce significant TC changes. Vertical lines define the different categories of neurones according to their TC; CS, cold-sensitive; IS, temperature-insensitive; WS, warm-sensitive. Concentrations used: muscimol, 0·1 and 1 μM; bicuculline, 5 μM.

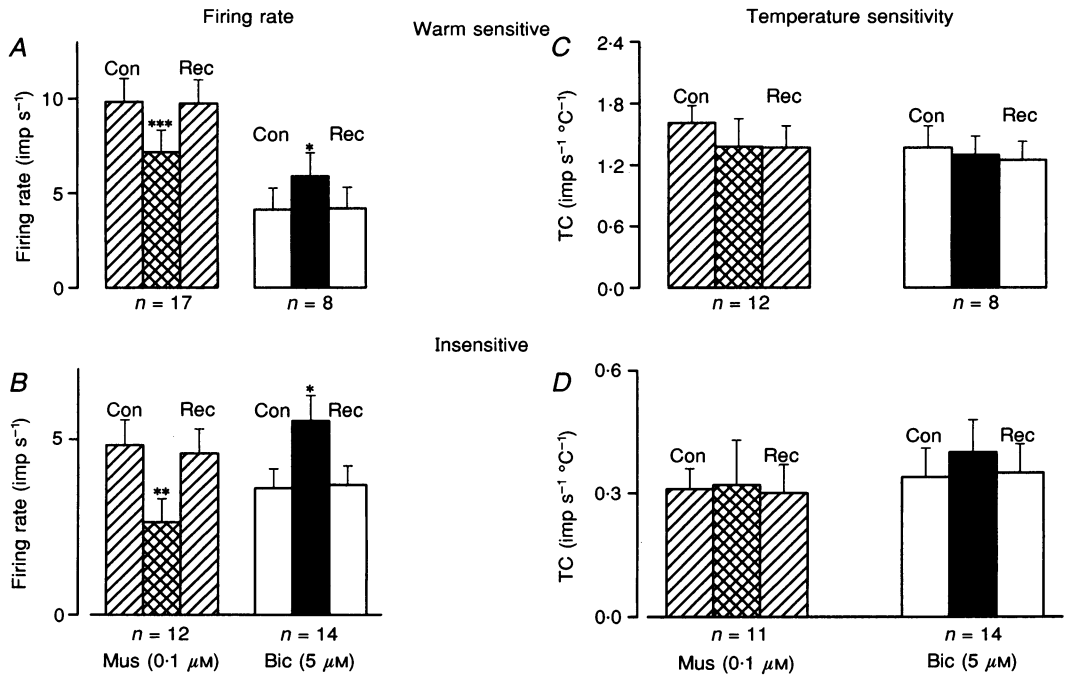

Figure 5. Effects of a $GABA_A$ agonist and antagonist on spontaneous activity and temperature sensitivity of warm-sensitive and temperature-insensitive hypothalamic neurones

Muscimol (Mus, 0·1 μM) decreased the mean firing rate of warm-sensitive and temperature-insensitive neurones while bicuculline (Bic, 5 μM) had the opposite effect (*A* and *B*). Neither muscimol nor bicuculline changed the mean temperature sensitivity of both categories of neurones (*C* and *D*). Effect of muscimol: ▨, mean values during the control (Con) and recovery (Rec) period; ▩, mean values during application. Effect of bicuculline: □, mean values during the control and recovery period; ■, mean values during application. Significant values: $*P < 0{\cdot}05$; $**P < 0{\cdot}01$; $***P < 0{\cdot}001$; means ± S.E.M.

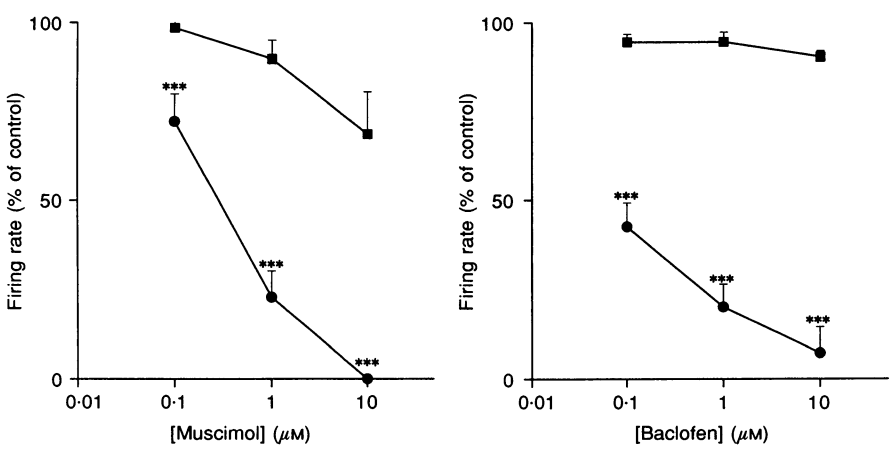

Figure 6. Dose–response relationships of the GABA agonists muscimol and baclofen on firing rate of neurones of the medial preoptic area

Data are presented as percentage of control firing rate (mean values ± S.E.M.). ●, data during drug application; ■, data obtained after wash out. Note that after a high dose of muscimol, recovery was incomplete. Significant values: $***P < 0{\cdot}001$.

Temperature sensitivity. In contrast to the obvious effect of muscimol on spontaneous activity, it did not affect the temperature sensitivity of the majority of neurones (Fig. 2) although the TC was increased or decreased in a few neurones (Fig. 4*A*). Often these changes were not reversible (Fig. 4*B*). The mean TC of the fifteen warm-sensitive neurones ($1{\cdot}59 \pm 0{\cdot}15 \rightarrow 1{\cdot}38 \pm 0{\cdot}25$ imp s^{-1} $°C^{-1}$) and fifteen temperature-insensitive neurones ($0{\cdot}32 \pm 0{\cdot}06 \rightarrow 0{\cdot}40 \pm 0{\cdot}12$ imp s^{-1} $°C^{-1}$) tested at muscimol concentrations of 0·1 and 1 μM was not significantly changed (see also Fig. 5). In six additional neurones the TC could not be estimated after drug application since their tonic activity was completely inhibited. The $GABA_A$ antagonist bicuculline also did not change the temperature sensitivity of hypothalamic neurones (Fig. 2) although it effectively increased the firing rate. Essentially, no deviation from the TC during the control period was observed at a concentration of 5 μM (Figs 4*C* and *D*; 5*C* and *D*). The temperature sensitivity was also not obviously altered by 10 μM bicuculline and consequently the mean TC for the twenty-two warm-sensitive neurones ($1{\cdot}40 \pm 0{\cdot}17 \rightarrow 1{\cdot}33 \pm 0{\cdot}19$ imp s^{-1} $°C^{-1}$) and thirty-six temperature-insensitive neurones ($0{\cdot}31 \pm 0{\cdot}07 \rightarrow 0{\cdot}34 \pm 0{\cdot}07$ imp s^{-1} $°C^{-1}$) tested with both concentrations used was not significantly changed.

Effects of substances acting on $GABA_B$ receptors

Spontaneous activity. Almost all of the neurones (36/37) in which the effect of the $GABA_B$ agonist baclofen (0·1, 1 and 10 μM) was tested, reacted with a dose-dependent decrease of tonic activity, regardless of their type of temperature sensitivity (Figs 6, 7 and 10). In contrast, to the relatively weak inhibitory effect of 0·1 μM muscimol, the same dose of baclofen reduced the average firing rate by more then 50% (Fig. 6). Furthermore, complete recovery was always observed, even after superfusion of 10 μM

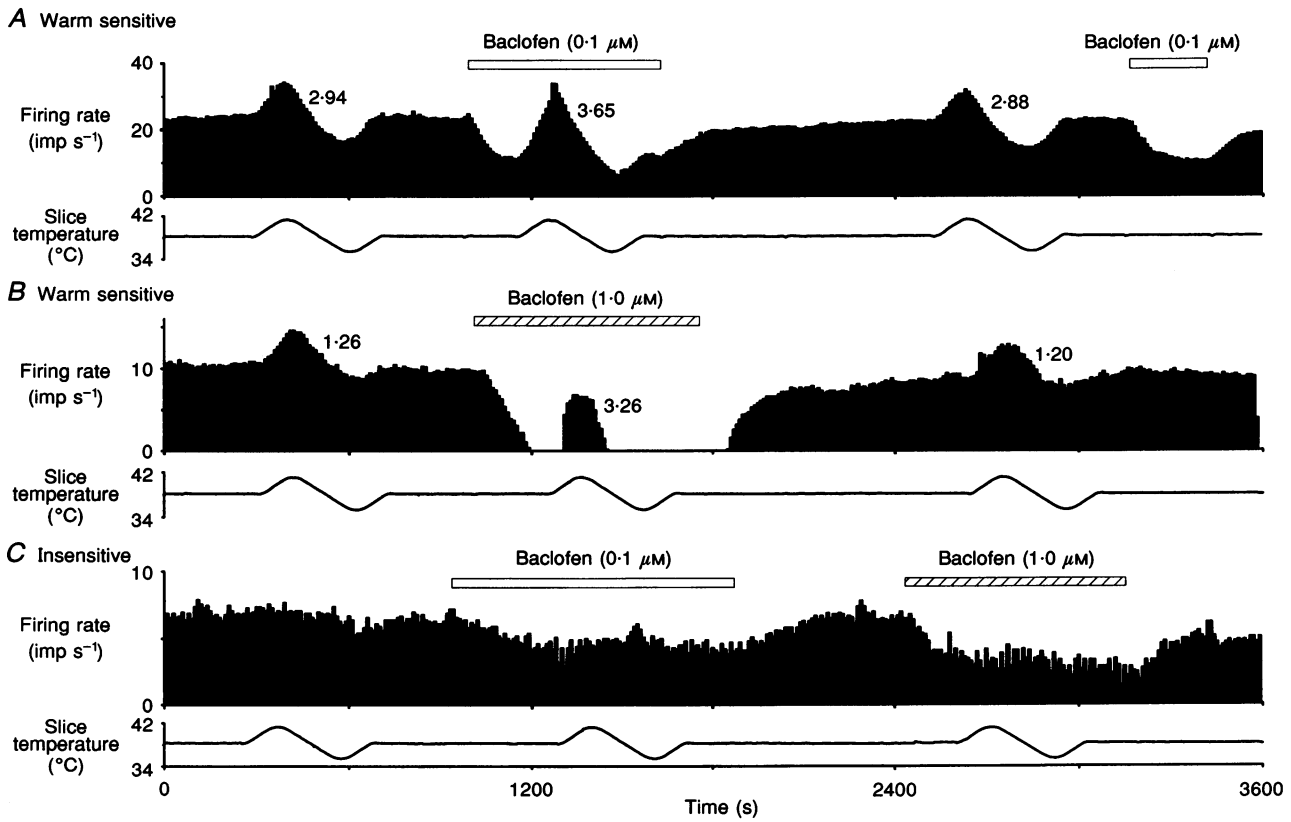

Figure 7. Effect of the $GABA_B$ agonist baclofen on spontaneous activity and temperature response of 3 different neurones of the medial preoptic area

Original recordings of firing rate and slice temperature from 2 warm-sensitive (*A* and *B*) and 1 temperature-insensitive (*C*) neurones. *A*, superfusion with baclofen (0·1 μM) lowered firing rate but also increased temperature sensitivity of a warm-sensitive neurone from 2·94 to 3·65 imp s^{-1} $°C^{-1}$. *B*, although a tenfold higher concentration of baclofen (1 μM) completely inhibited tonic activity, the neurone still reacted to the temperature stimulus with a TC of almost 3 times the control value (from 1·26 to 3·26 imp s^{-1} $°C^{-1}$). The calculated TC for a given temperature stimulus is indicated at the responses for the two warm-sensitive neurones. *C*, baclofen also reduced dose-dependently the firing rate of a temperature-insensitive neurone but did not affect temperature sensitivity.

baclofen. No concentration dependency was found for the duration of the response, since the delay to the maximal effect after onset of superfusion (2·1–3·0 min) as well as the beginning of restoration after drug administration was stopped (3·5–3·9 min) was notably constant for the different concentrations used (Table 2).

Essentially no effect on firing rate was seen in the twenty-seven warm-sensitive and temperature-insensitive neurones tested after superfusion of the $GABA_B$ antagonist phaclofen at the low concentration of 10 μM (Figs 8 and 10). However, superfusion of 100 μM phaclofen induced a modest but significant ($P < 0{\cdot}05$) increase in spontaneous activity ($n = 12$) from 5·65 ± 0·85 to 7·28 ± 1·73 imp s^{-1} (Fig. 8*C*). The observation that doses of 100 μM phaclofen were necessary to block the inhibition produced by baclofen confirms the notion that phaclofen affects spontaneous activity only in high doses (Fig. 8*C*). Nevertheless this antagonistic effect appears to be specific since the inhibition induced by the $GABA_A$ agonist muscimol was not affected by phaclofen.

Temperature sensitivity. Both the $GABA_B$ agonist as well as the antagonist specifically altered the TC of warm-sensitive neurones, while the TC of temperature-insensitive neurones was not significantly changed (Figs 7, 9 and 10). The $GABA_B$ agonist baclofen at a concentration of 0·1 μM increased the TC of warm-sensitive neurones by 0·61 ± 0·11 imp s^{-1} $°C^{-1}$ ($n = 9$); this effect was slightly more pronounced at a concentration of 1 μM (0·72 ± 0·13 imp s^{-1} $°C^{-1}$; $n = 9$). The augmentation of temperature sensitivity was so profound that even when the spontaneous activity was completely inhibited by the drug, the neurone could be activated by a temperature stimulus (Fig. 7*B*).

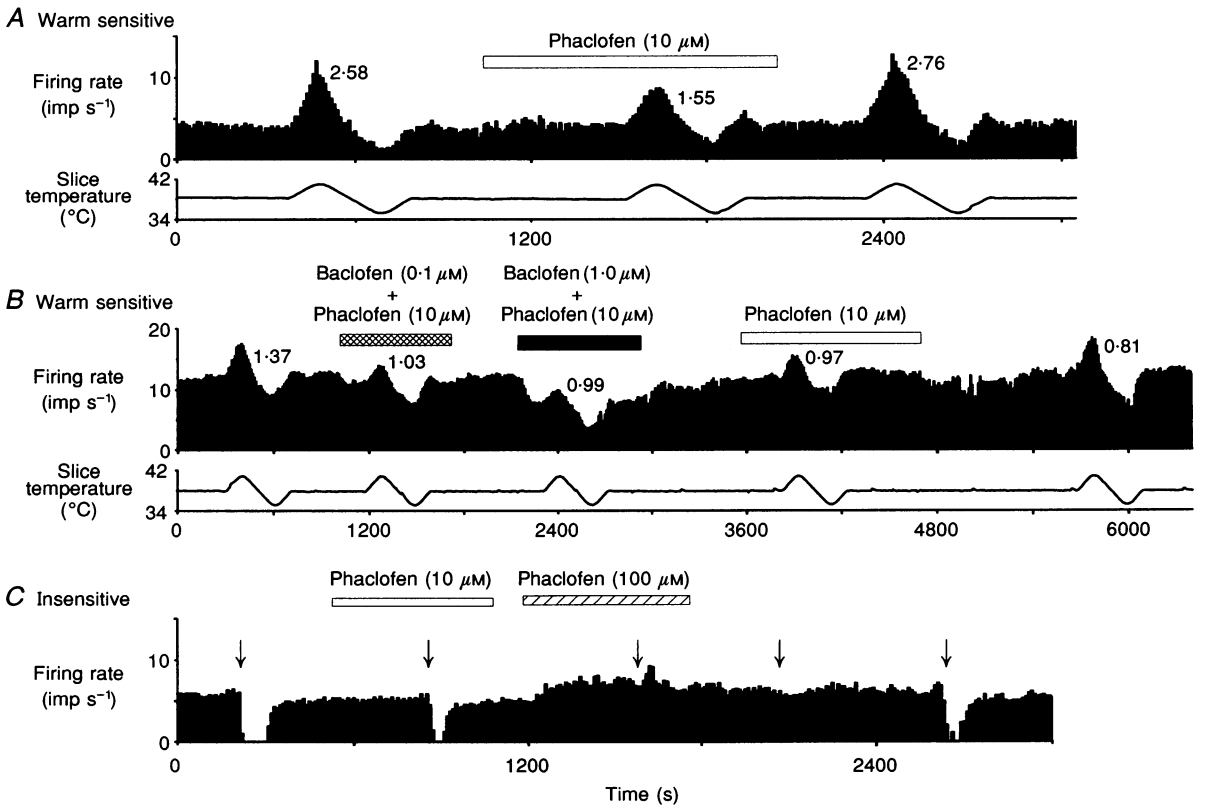

Figure 8. Effects of a $GABA_B$ agonist and antagonist on spontaneous activity and temperature response of 3 different neurones from the medial preoptic area

Original recordings of the firing rate and slice temperature of 2 warm-sensitive (*A* and *B*) and 1 temperature-insensitive neurone (*C*). *A*, superfusion with phaclofen did not affect the firing rate but decreased temperature sensitivity (from 2·58 to 1·55 imp s^{-1} $°C^{-1}$). *B*, during combined superfusion phaclofen did not affect the baclofen action on firing rate but decreased the temperature sensitivity from 1·37 to 1·03 (0·1 μM baclofen) and 0·99 imp s^{-1} $°C^{-1}$ (1·0 μM baclofen). Note that phaclofen alone reduced the TC to a similar degree (0·97 imp s^{-1} $°C^{-1}$) and that the temperature sensitivity completely recovered after wash out. The calculated TC for a given temperature stimulus is indicated at the responses for the two warm-sensitive neurones. *C*, superfusion of phaclofen reduced (10 μM) or prevented (100 μM) the inhibitory effect of bolus injections of baclofen (arrows, 0·1 ml of 1 μM) on spontaneous activity and even increased the firing rate of a temperature-insensitive neurone at the high concentration (100 μM). Note recovery of the inhibitory effect of baclofen.

The specificity of the effect of $GABA_B$ergic substances is confirmed by the finding that the $GABA_B$ antagonist phaclofen has the opposite effect on the temperature sensitivity of warm-sensitive hypothalamic neurones. At the concentration of 10 μM, which did not affect spontaneous activity, phaclofen decreased the TC of all warm-sensitive neurones tested (Figs 8, 9 and 10). This decrease was large enough to convert three of the neurones to temperature-insensitive ones. It should be noted that the TC changes induced by baclofen and phaclofen were completely reversed after washing with ACSF (Figs 9 and 10). The baclofen-induced increase in TC could be entirely prevented by 10 μM phaclofen. When phaclofen (10 μM) was co-perfused with 0·1 or 1 μM baclofen the decrease of the TC was similar to the decrease induced by phaclofen alone (Fig. 8*B*), indicating that this low dose of the antagonist is able to block the effects of intrinsic and extrinsic GABA agonists on temperature sensitivity acting through $GABA_B$ receptors.

Both substances affected the TC of the cold-sensitive neurones tested (1 with baclofen and 2 with phaclofen) in a fashion similar to warm-sensitive neurones, i.e. baclofen increased but phaclofen decreased the TC. The change of the TC induced by baclofen was high enough to convert this neurone into a temperature-insensitive one. The effect on spontaneous activity resembled that on warm-sensitive neurones: baclofen inhibited the spontaneous activity while phaclofen had no effect on firing rate.

$GABA_A$ and $GABA_B$ receptor interaction with regard to temperature sensitivity of PO/AH neurones

It has been demonstrated that pretreatment with the $GABA_A$ antagonist bicuculline unmasks or pronounces the hypothermia induced by the $GABA_B$ agonist baclofen (Sancibrian, Serrano & Minano, 1991). To test the possible interaction of $GABA_A$ and $GABA_B$ mechanisms bicuculline and baclofen were sequentially applied to eight warm-

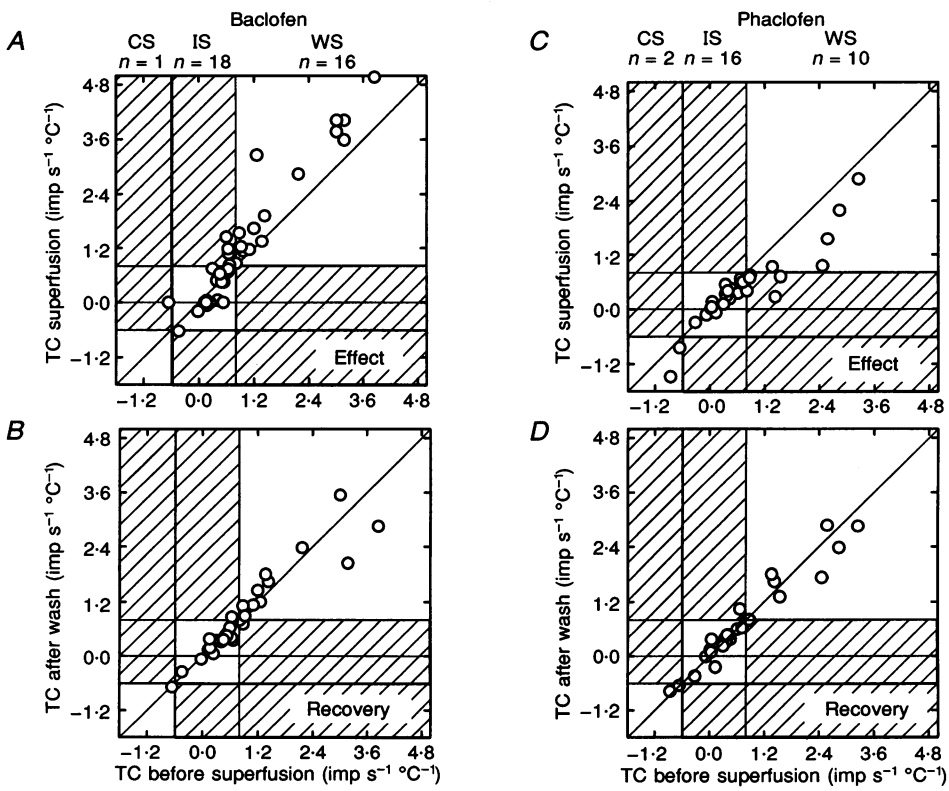

Figure 9. Effects of the $GABA_B$ agonist baclofen and antagonist phaclofen on temperature sensitivity of hypothalamic warm-sensitive neurones

To illustrate the TC changes of individual neurones the TC during application (effect) of baclofen (*A*) and phaclofen (*C*) and after washing (*B*, *D*, recovery) is plotted as a function of the TC during the control period. The distance of a circle from the line of identity indicates the degree of change. Circles in hatched areas represent neurones in which the TC changes were large enough to transform this neurone into another category. Baclofen increased and phaclofen decreased the temperature sensitivity of warm-sensitive neurones while temperature-insensitive neurones were not affected; phaclofen also reduced the TC of 2 cold-sensitive neurones. Vertical lines define the different categories of neurones according to their TC; CS, cold-sensitive; IS, temperature-insensitive; WS, warm-sensitive. Concentrations used: baclofen, 0·1 and 1 μM; phaclofen, 10 μM.

sensitive and four temperature-insensitive neurones. All neurones reacted to baclofen with a decrease of tonic activity while only six warm-sensitive and one temperature-insensitive reacted to bicuculline. In these six warm-sensitive neurones co-perfusion of bicuculline and baclofen accentuated the effect of baclofen on temperature sensitivity by increasing the TC by 0·74 ± 0·11 imp s^{-1} $°C^{-1}$ compared with the increase of 0·35 ± 0·10 imp s^{-1} $°C^{-1}$ induced by baclofen alone. The TC of the four temperature-insensitive neurones was not changed by bicuculline or baclofen nor by co-perfusion of these two substances.

DISCUSSION

Effect of GABAergic substances on the firing rate

Our present results demonstrate that $GABA_A$ as well as $GABA_B$ agonists reduce the activity of both warm-sensitive and temperature-insensitive PO/AH neurones, which conforms to the general depressive effect of GABA in the central nervous system. This effect is specific since it is blocked *only* by the appropriate antagonists. This is in agreement with the demonstration of $GABA_A$ and $GABA_B$ receptors in the hypothalamus (Palacios, Wamsley & Kuhar, 1981; Gehlert, Yamamura & Wamsley, 1985).

The neuronal network in the anterior hypothalamus appears to be under tonic control of continuously released GABA, since the $GABA_A$ antagonist bicuculline as well as the $GABA_B$ antagonist phaclofen (although the latter only at the high concentration of 100 μM) increase the spontaneous activity of warm-sensitive as well as temperature-insensitive neurones. The relatively high doses of phaclofen necessary to block the baclofen-induced inhibition has also been reported by others (Kerr, Ong, Prager, Gynther & Curtis, 1987).

The morphological substrates for this tonic inhibition are GABA-containing small and medium size neurones that have been demonstrated in different areas of the hypothalamus (Ottersen & Storm-Mathisen, 1984; Decavel & Van den Pol, 1990; Okamura *et al.* 1990). Furthermore, spontaneous GABA release could be demonstrated in the PO/AH *in vivo* (Jarry, Perschl & Wuttke, 1988).

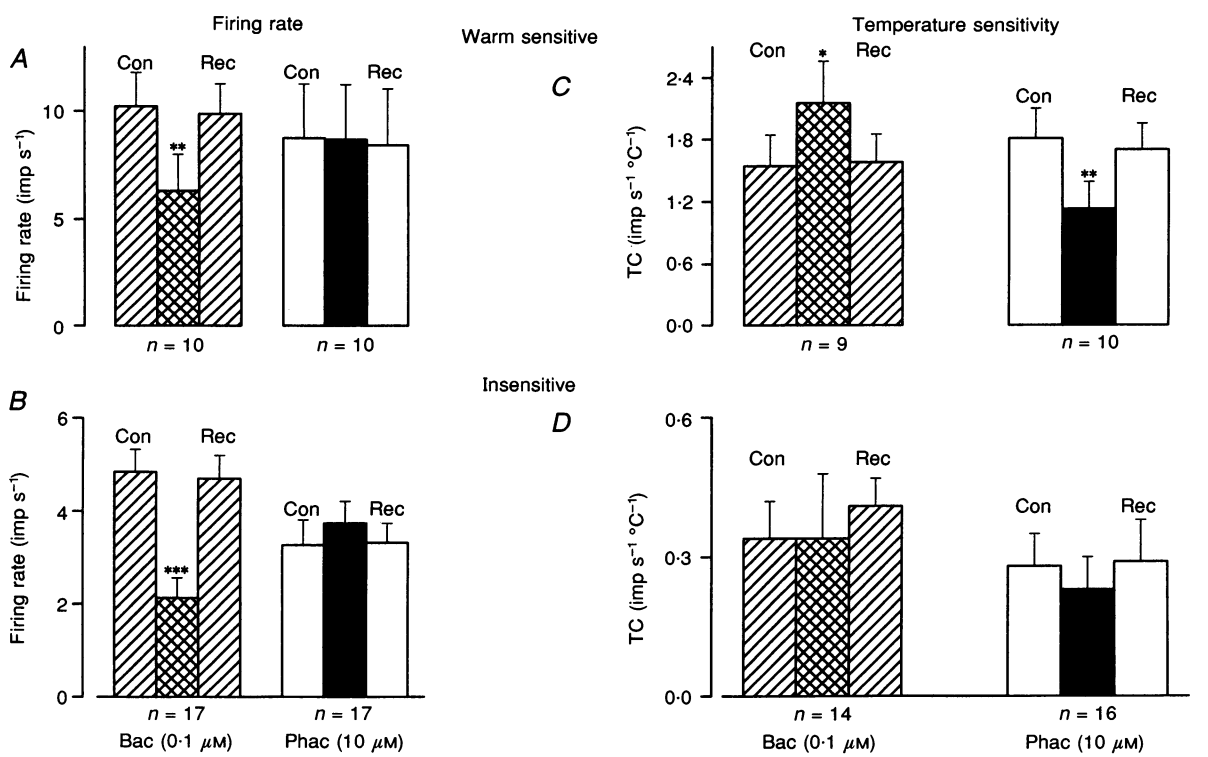

Figure 10. Effects of the $GABA_B$ agonist baclofen and the antagonist phaclofen on spontaneous activity and temperature sensitivity of warm-sensitive and temperature-insensitive hypothalamic neurones

Baclofen (Bac, 0·1 μM) decreased the mean firing rate of warm-sensitive (*A*) and temperature-insensitive neurones (*B*) while phaclofen (Phac, 10 μM) had no effect. Baclofen increased and phaclofen decreased the temperature sensitivity of warm-sensitive neurones (*C*) while the effect on temperature-insensitive neurones was not significant (*D*). Effect of baclofen: ▨, mean values during the control (Con) and recovery (Rec) period, respectively; ▩, mean values during application. Effect of phaclofen: □, mean values during the control and recovery period, respectively; ■, mean values during application. Significant values: $*P < 0·05$; $**P < 0·01$; $***P < 0·001$; means ± S.E.M.

Intracellular recordings have revealed frequent spontaneous inhibitory postsynaptic potentials (IPSPs) in warm-sensitive and cold-sensitive hypothalamic neurones (Curras, Kelso & Boulant, 1991) and these IPSPs are most probably mediated by GABA (Hoffman, Wuarin & Dudek, 1994). The small spikes recruited during bicuculline superfusion in about one-third of the neurones recorded in the present investigation also indicate that the spontaneous activity of a number of neurones is inhibited via GABAergic mechanisms. A similar percentage of neurones was found to be silent in the investigation of Curras *et al.* (1991).

Effects of GABAergic substances on temperature sensitivity

Particularly $GABA_B$ mechanisms appear to modify the temperature sensitivity of warm-sensitive PO/AH neurones. Most noticeably the $GABA_B$ agonist baclofen increased the response to temperature changes, i.e. temperature sensitivity, although the tonic firing rate was decreased. The specificity of the baclofen-induced increase of the TC is substantiated by the opposite effect of the $GABA_B$ antagonist phaclofen. These observations conform to the hypothesis that modulation of the temperature sensitivity of hypothalamic neurones is involved in the control of body temperature under physiological conditions (Blatteis, 1981).

The specific effect of $GABA_B$ agonists on the temperature sensitivity of warm-sensitive neurones should be preferentially due to postsynaptic rather than presynaptic mechanisms since the warm sensitivity of hypothalamic neurones appears to be largely independent from excitatory synaptic input (Curras *et al.* 1991). On postsynaptic neurones the $GABA_B$ receptor-mediated increase in K^+ conductance generates fast and slow IPSPs. It has been demonstrated by Curras *et al.* (1991) that IPSPs, most probably of GABAergic origin, can increase the interspike interval and that this ability is augmented by cooling, i.e. warm sensitivity can be modulated by inhibitory synaptic input. Consequently, the promotion of GABAergic postsynaptic potentials by $GABA_B$ agonists could increase warm sensitivity of hypothalamic neurones while $GABA_B$ antagonists could produce the opposite effect. In addition, slow GABAergic IPSPs might facilitate discharge frequency by removal of the inactivation of low voltage-activated Ca^{2+} currents (Crunelli & Leresche, 1991); however, it is not known whether this facilitation is modulated by temperature changes. On the other hand, shortening of action potentials and decrease of after-hyperpolarization with increasing temperatures appear to contribute to neuronal temperature sensitivity (Pierau, Klee & Klussmann, 1976; Curras *et al.* 1991). The shortening of action potentials due to an inhibition of Ca^{2+} currents and the subsequent reduction of after-hyperpolarization due to a reduced Ca^{2+}-activated K^+ current by $GABA_B$ receptor activation observed by several investigators (see Matsushima, Tegner, Hill & Grillner, 1993) might promote temperature sensitivity.

Although the cellular mechanism of the temperature sensitivity of hypothalamic neurones is not completely understood, intracellular recordings suggest that action potentials of warm-sensitive neurones are preceded by pre-potentials or pacemaker potentials and that the rate of rise of these depolarizing prepotentials increases with warming (Curras *et al.* 1991). Modulation of the prepotentials could be a possible mechanism by which $GABA_B$ receptor activation changes temperature sensitivity of warm-sensitive hypothalamic neurones. One of the modulating factors of depolarizing pre-potentials is the fast activating and inactivating A current (Connor & Stevens, 1971). The inactivation of the A current was particularly sensitive to temperature changes in T lymphocytes resulting in an increase of A current inactivation in the hyperthermic temperature range (Pahapill & Schlichter, 1990). Such an increase of A current inactivation would tend to increase the slope of depolarizing prepotentials. It is not known whether $GABA_B$ agonist or antagonists affect the inactivation rate of A currents. But it has been demonstrated that baclofen shifts the voltage dependence of the activation in a depolarizing direction (Saint, Thomas & Gage, 1990), which would rather decrease the prepotential slope. Thus, it remains unresolved whether the effect of $GABA_B$ substances on A current parameters contribute to their modulatory effect on neuronal temperature sensitivity.

Other K^+ channels might also contribute to the temperature dependence of warm-sensitive neurones. It has been observed by Curras *et al.* (1991) that the current–voltage relationship of some warm-sensitive neurones demonstrates an inward rectification at neutral and cool temperatures, but not at warm temperatures. Therefore modulation of the inward rectifier by $GABA_B$ receptor activation has also to be considered to participate in $GABA_B$-produced increase of temperature sensitivity, since the K^+ current induced by $GABA_B$ agonists demonstrates inward rectification (Newberry & Nicoll, 1985).

Effect of GABA on temperature regulation

Central and systemic application of GABA and GABA agonists usually causes a fall in core temperature, while the antagonists induce hyperthermia (Clark & Lipton, 1985; Serrano *et al.* 1985; Minano, Serrano, Sancibrian & Myers, 1989; Yakimova & Ovtcharov, 1989; Jackson & Nutt, 1991). However, the intensity, duration and direction of the thermoregulatory changes depend on the route and dose of application, animal species, ambient temperature and general conditions, e.g. restraint of movement etc.

The first indication that the GABA effect might be receptor specific came from the experiments of Serrano *et al.* (1985), which demonstrated that the GABA-induced hypothermia is not blocked by bicuculline, suggesting that the GABA effect on thermoregulation is not primarily mediated through activation of $GABA_A$ receptors. Since intraperitoneal, as

well as intraventricular, application of baclofen in doses between 5–10 mg kg^{-1} (I.P.) and 5–15 ng (I.C.V.) induced hypothermia in mice it was suggested that $GABA_B$ receptor stimulation is important for the hypothermic effect of GABA (Gray, Goodwin, Heal & Green, 1987; Jackson & Nutt, 1991). However, $GABA_A$ receptors may also participate in thermoregulation since pretreatment with the $GABA_A$ antagonist bicuculline (3 mg kg^{-1} I.P.) augments the hypothermia induced by low doses of baclofen (1–10 mg kg^{-1} I.P.) in *restrained* rats (Sancribian *et al.* 1991). The antagonistic interaction of $GABA_A$ and $GABA_B$ receptors in thermoregulatory responses might be due to a modulating effect of $GABA_A$ on $GABA_B$ receptors as is indicated by the augmentation of the baclofen-induced increase of temperature sensitivity of warm-sensitive hypothalamic neurones in the presented experiments.

Interaction of $GABA_B$ receptors with other receptor mechanisms may also play a role in GABA-mediated changes of body temperature; e.g. higher doses of baclofen (30 mg kg^{-1} I.P.) produced an initial temperature fall (30 min) but then increased body temperature, probably due to activation of opioid and/or prostaglandin-mediated mechanisms (Sancribian *et al.* 1991). An increase in body temperature due to activation of brown adipose tissue was also seen after intraventricular administration of 1 μg baclofen in *anaesthetized* rats (Horton, LeFeuvre, Rothwell & Stock, 1988). This hyperthermia was abolished by simultaneous application of the $GABA_A$ agonist muscimol, again indicating $GABA_A$ and $GABA_B$ receptor interaction.

Models of temperature regulation

The question arises as to whether the different effects of GABAergic substances on hypothalamic neurones correspond to the changes in temperature regulation observed. Previous studies have shown that various neurotransmitters and neuromodulators which affect temperature regulation by intrahypothalamic or intraventricular application may change the activity of PO/AH neurones (Cabanac, Stolwijk & Hardy, 1968; Eisenman, 1969; Boulant, 1980; Hori, 1991). Under *in vivo* as well as *in vitro* conditions it was observed that substances causing hypothermia, such as bombesin and capsaicin, usually enhance tonic activity of warm-sensitive PO/AH neurones (Hori, Shibata, Kiyohara, Nakashima & Asami, 1988; Schmid, Jansky & Pierau, 1993) while those leading to hyperthermia, such as pyrogens, interferon-α, prostaglandin E_2, have a depressing effect (Cabanac *et al.* 1968; Eisenman, 1969; Nakashima, Hori, Kuriyama & Matsuda, 1988). Spontaneous activity of temperature-insensitive neurones was also enhanced by the hypothermic substances bombesin and capsaicin but was little or not affected by hyperthermic substances. This is in agreement with neuronal models of hypothalamic control of body temperature which predict that an increased activity of warm-sensitive neurones causes hypothermia by activating heat loss mechanisms, while the reduction of firing rate of warm-sensitive neurones activates mechanisms for heat conservation and heat production (Bligh, 1981).

However, the observed decrease of spontaneous activity of warm-sensitive hypothalamic neurones after application of GABA agonists does not fit to the model which would predict that substances causing hypothermia increase the tonic activity. Recent experiments have revealed that the effect on spontaneous activity might not be the main indicator for a specific action of a substance on PO/AH neurones (Schmid & Pierau, 1990). For example, bombesin and substance P both increase the spontaneous activity of temperature-sensitive hypothalamic neurones (Schmid *et al.* 1993) but only bombesin considerably decreases body temperature by intrahypothalamic application (Jansky, Riedel, Simon, Simon-Oppermann & Vybiral, 1987) while substance P either has no effect or causes a small increase of body temperature. What differentiates the two types of substances is the effect on the temperature sensitivity of PO/AH neurones. Bombesin increases the TC of the majority of warm-sensitive neurones and of almost all temperature-insensitive neurones. The effect on the latter leads to a recruitment of warm-sensitive neurones resulting in an increase in the signal output of the warm pathway to the effector neurones. Substance P on the other hand decreases the temperature sensitivity of most of the warm-sensitive neurones but has very little effect on the TC of temperature-insensitive neurones. The hypothesis was put forward that a substance which decreases body temperature increases the temperature sensitivity of PO/AH neurones while a decrease of the TC is characteristic for hyperthermic substances. This thesis has been confirmed for other substances affecting thermoregulation (Pierau *et al.* 1994).

The $GABA_B$ substances fulfil the criteria of the above hypothesis since the temperature sensitivity of warm-sensitive PO/AH neurones is significantly increased by the $GABA_B$ agonist baclofen and decreased by the $GABA_B$ antagonist phaclofen. However, in contrast to bombesin, the TC of temperature-insensitive neurones was not significantly changed, although there was a tendency for a decrease, and consequently no recruitment of warm-sensitive neurones was obtained by baclofen. Our result showing that only $GABA_B$ substances significantly change the temperature sensitivity of warm-sensitive neurones is in agreement with the notion that the GABA effect on temperature regulation is preferentially mediated by $GABA_B$ receptors (Serrano *et al.* 1985; Jackson & Nutt, 1991). The observation that the $GABA_A$ antagonist bicuculline augments the increase in the temperature sensitivity of hypothalamic neurones induced by the $GABA_B$ agonist baclofen offers a possible explanation for the amplification of the baclofen-induced hypothermia by the $GABA_A$ antagonist bicuculline observed in restrained rats (Sancibrian *et al.* 1991).

The presented results support the hypothesis that changes in temperature sensitivity of hypothalamic neurones rather than an effect on their tonic activity are connected to the hypo- or hyperthermic action of a substance. The action of a drug on spontaneous activity of PO/AH neurones is, however, also likely to contribute to its effect on body temperature. Presently it remains unresolved as to how the two parameters interact. In addition, the interaction of pre- and postsynaptic $GABA_A$ and $GABA_B$ pathways, as well as the possible activation of other mediators such as opioids or prostaglandins by GABAergic substances, suggests a high degree of complexity in the neuronal network involved in temperature regulation.

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