

THE EFFECT OF BACLOFEN AND SOMATOSTATIN ON NEURONAL ACTIVITY IN THE RAT VENTROMEDIAL HYPOTHALAMIC NUCLEUS *IN VITRO*

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Summary—The electrical properties of neurones within the ventromedial hypothalamic nucleus of the rat were studied in an *in vitro* slice preparation, using conventional intracellular recording techniques. A detailed analysis of 36 intracellular recordings appeared to suggest 3 cell types, based on membrane capacitance and resistance characteristics, confirming previous reports of a diversity of cell types within this nucleus. The responsiveness of each cell type to exogenously-applied baclofen and somatostatin was also investigated. The inhibitory responses to both of these drugs were concentration-related (over the range 100 nM to 1 μ M), tetrodotoxin-resistant and consisted of a membrane hyperpolarization (mean \pm SEM = 6.7 ± 1 and 10.7 ± 1 mV for 1 μ M somatostatin and baclofen, respectively) and an associated reduction in the firing frequency of spontaneously active cells. These agonist-evoked responses probably represented direct postsynaptic actions but they were not restricted to any single type of cell. Evidence for an additional presynaptic effect of baclofen was also obtained. Responses to baclofen were extremely robust and readily quantifiable, whereas those to somatostatin showed pronounced long-lasting desensitization, which was particularly marked at larger concentrations. These data support previous contentions, based on *in vivo* studies, that somatostatin and GABA are likely to participate in the control of complex functions by the ventromedial hypothalamic nucleus.

Key words—ventromedial hypothalamus, somatostatin, baclofen, GABA, electrophysiology, brain slice, receptors.

The ventromedial nucleus, largest of the tuberal nuclei in the mediobasal hypothalamus, is thought to participate in a number of higher functions of the brain including aggression and emotional behaviour, feeding and obesity and the regulation of endocrine hypophyseal secretions (see Morgane and Panksepp, 1980). γ -Aminobutyric acid (GABA) and somatostatin are among the many aminergic and peptidergic transmitters that have been implicated in one or more of these activities by an action involving the ventromedial hypothalamic nucleus. Interest in GABA began with an early demonstration of an avid uptake mechanism for the tritiated amino acid (Makara, Rappay and Stark, 1975) and more recently by *in vivo* experiments, which have suggested a role for GABA_A receptors in the regulation of food intake (Kamatchi, Veeraragavan, Chandra and Bapna, 1986) and affective behaviour (Hansen and Ferreira, 1986). Preliminary observations from this laboratory (Newberry and Priestley, 1988) demonstrated that the selective GABA_A receptor agonist, isoguvacine, had powerful inhibitory actions on extracellularly-recorded single unit activity in the ventromedial hypothalamic nucleus. Although there has been no demonstration of GABA_B binding sites within the ventromedial hypothalamic nucleus, there is evidence to suggest that GABA_B receptors, within this nucleus, may be involved in thermogenesis (Addae, Rothwell, Stock and

Stone, 1986) and in the regulation of gastric motility (Wood, Addae, Andrews and Stone, 1987).

Somatostatin is a tetradecapeptide which is perhaps best known for its regulation of growth hormone secretion, an action which may, at least in part, be mediated within the ventromedial hypothalamic nucleus (Martin, 1972; Bernardis and Frohman, 1971). The peptide has also been found to affect feeding behaviour (Ho, Chern and Lin, 1989), by an action involving the ventromedial hypothalamic nucleus. These reports are supported by the demonstration of somatostatin-like immunoreactivity in axons and axon terminals within the ventromedial hypothalamic nucleus (Hokfelt, Efendic, Johannsen, Luft and Arimura, 1974; Bennett-Clarke, Romagnano and Joseph, 1980) and the presence of somatostatin mRNA within neurones of the ventromedial hypothalamic nucleus (Kiyama and Emson, 1990). However, autoradiographic studies of the hypothalamus have consistently demonstrated only a very sparse density of binding sites within the ventromedial hypothalamic nucleus, for radiolabelled somatostatin peptides (Uhl, Tran, Martin and Snyder, 1985; Leroux, Gonzalez, Laquerriere, Bodenat and Vaudry, 1988; Krantic, Martel, Weissman and Quirion, 1989).

The ability of a single nucleus to apparently influence such diverse aspects of body function has led to

the idea of specialized cells within the ventromedial hypothalamic nucleus. Indeed, several detailed cytological studies of this nucleus have consistently demonstrated the presence of morphologically different cell types (Takeichi and Noda, 1974; Millhouse, 1978; Van Houten and Brawer, 1978), observations which have been substantiated by the identification of 2 (Murphy and Renaud, 1969; Boden and Hill, 1988) or 3 (Minami, Oomura and Sugimori, 1986) cell types, based on electrophysiological characteristics. In support of specialization, it has recently been demonstrated that the effects of cholecystokinin (CCK) are restricted to a particular subset of neurones in the ventromedial hypothalamic nucleus (Boden and Hill, 1988).

The specific aim of the present study was to determine whether two pharmacologically unrelated substances, such as baclofen and somatostatin, which nevertheless have mechanistically very similar membrane effects, act on clearly identifiable cell types, within a single hypothalamic nucleus. Particularly interesting in this regard would be the demonstration that some or all of the behavioural effects of baclofen and somatostatin were the result of an action on neurones with demonstrably different electrophysiological characteristics. With this in mind, the following account describes a detailed quantitative evaluation of the intracellular effects of the GABA_B agonist, baclofen (β -(*p*-chlorophenyl)GABA) and of somatostatin in an *in vitro* slice preparation of the ventromedial hypothalamic nucleus.

METHODS

Preparation of slices of hypothalamus

Male Sprague–Dawley rats (80–130 g) were decapitated and the brain rapidly removed. A block of tissue (approx 5 mm), containing the ventromedial hypothalamic nucleus, was cut from the brain using a razor blade. This was then glued onto a glass slide, using cyanoacrylate cement and transferred to the submerged stage of an Oxford Vibratome. The tissue block was submerged in a salt solution of the following composition (mM): NaCl, 124; KCl, 2; KH₂PO₄, 1.25; MgCl₂, 2; CaCl₂, 2; NaHCO₃, 25; D-glucose, 11. The solution was continuously gassed with a mixture of 95% O₂/5% CO₂ to maintain a buffered pH of 7.4. Slices were cut from the block of tissue at a thickness of 350 μ m and, after additional trimming, were transferred to a similarly gassed holding chamber. Electrophysiological recordings were performed on individual slices, which were mounted on a submerged nylon gauze platform within a perspex recording chamber (approx volume 0.3 ml) and continuously perfused (approx 1 ml min⁻¹) with the same gassed salt solution described above but at a temperature of 33°C. Drugs were applied to the slice by bath perfusion.

Intracellular recordings

Intracellular recordings were performed using an Axoclamp 2A amplifier, signals were filtered at a cut-off frequency of 3 kHz. Electrodes were pulled from borosilicate or aluminosilicate glass (GC120F and SM100F, respectively, Clark Electromedical), using a Brown Flaming puller (Sutter instruments); they were backfilled with either 3M KCl or 3M KAc. Electrode resistances varied according to the electrolyte used but generally were between 50–100 M Ω . Electrodes which showed excessive rectification or a tendency to block after impalement were discarded. Digitized signals were recorded on tape and subsequently analysed off-line using SCAN software (J. Dempster, University of Strathclyde, U.K.). Input resistances of the cells were calculated from the linear regression of current–voltage relationships, obtained by injecting hyperpolarizing current pulses of varying amplitudes into the cell and measuring the resulting voltage deflection.

Drugs

Somatostatin-14 was purchased from Bachem U.K., dissolved as a 1 mM stock solution in distilled water, aliquotted and frozen at –30°C until required, a fresh aliquot was used for each experiment. (–)Baclofen was purchased from Research Biochemicals Inc.

RESULTS

Intracellular recordings were made from a total of 36 neurones within the ventromedial hypothalamic nucleus, 28 of these cells were studied in greater detail, the results of this analysis appeared to indicate 3 types of cell with differing electrophysiological profiles. The 3 categories of cells were differentiated primarily on their membrane time constants. The properties of the 3 cell types are summarized in Table 1. Time constants were determined from the exponential function, fitted to the time taken to fully charge the membrane capacitance, following the injection of hyperpolarizing electrotonic current pulses. Type 1 cells were generally quiescent cells which, apart from immediately following penetration, showed little tendency to fire spontaneous action potentials but occasionally showed anode-break spikes (Fig. 1). These cells also had smaller membrane input resistances, shorter membrane time constants and shorter afterhyperpolarizations than the other categories; they were also the most infrequently encountered of the 3 types of cell, representing only 11% of cells in the present study. Type 2 cells were defined as being spontaneously active, having prominent, lengthy afterhyperpolarizations (Fig. 1), long membrane time constants and large input resistances. Type 3 cells resembled Type 2 cells in many of their characteristics (Fig. 1). They differed in that they had significantly longer membrane time constants, large input resistances and were usually spontaneously active. In

Table 1. Analysis of action potential characteristics

Cell type	Spike amplitude (mV)	Membrane time constant (msec)	Firing frequency (Hz)	AHP		Input resistance (M Ω)	Membrane potential (mV)	n
				Ampl. (mV)	Dur. (msec)			
1	54 \pm 7	11 \pm 3	0	12 \pm 1	61 \pm 20	180 \pm 21	59 \pm 1	3
2	66 \pm 3	18 \pm 1	8 \pm 1	13 \pm 1	109 \pm 17	290 \pm 34	57 \pm 2	11
3	69 \pm 3	34 \pm 2*	9 \pm 3	11 \pm 1	207 \pm 37	400 \pm 31†	63 \pm 2	14

Data represent means \pm SEM, where n = the number of cells studied.

AHP = Spike afterhyperpolarization, Ampl. = amplitude, Dur. = duration.

*Significantly different from Types 1 and 2, $P < 0.02$. †Significantly different from Types 1 and 2, $P < 0.05$.

approximately 45% of cases, the afterhyperpolarizations of Types 2 and 3 cells comprized both fast and slow components. In Types 2 and 3 cells the large membrane resistance conferred a high sensitivity of action potential frequency to the membrane potential, such that the injection of small hyperpolarizing d.c. currents of constant amplitude (0.01 nA, the smallest current possible with the Axoclamp HS2 headstage), resulted in clear reductions in spike fre-

quency (not shown). The majority of recorded cells in these 2 categories showed a high frequency of post-synaptic potentials (PSP's), regardless of whether KCl or KAc was used within the electrode, this activity persisted after treatment with tetrodotoxin (TTX) treatment but was abolished by bicuculline (10 μ M, $n = 3$ cells) (Fig. 2).

Baclofen produced concentration-related hyperpolarizations (Fig. 4) in all Types 1, 2 and 3 neurones of the ventromedial hypothalamic nucleus tested (19 cells). In non TTX-treated Types 2 and 3 cells, this was always associated with a concentration-related reduction in frequency of action potentials (Fig. 3), there were also occasions where small concentrations of baclofen did not produce an overt effect on the membrane potential but still resulted in an obvious decline in spike frequency. Hyperpolarizations, produced by 1 μ M or greater concentrations of baclofen were almost always associated with a significant decrease in cell input resistance (e.g. Fig. 3). The effects of baclofen persisted in TTX-treated cells and the GABA_B agonist appeared to reduce the frequency of TTX-resistant PSP's, whenever present (Fig. 2).

The effects of somatostatin were more variable than those of baclofen but likewise were not restricted to any one category of cell. When applied at 1 μ M the peptide hyperpolarized all of the neurones of the ventromedial hypothalamic nucleus which were tested (12 cells, e.g. Fig. 3), as with baclofen this was associated with a reduction in the frequency of action potentials in non TTX-treated Types 2 and 3 cells, although the effect was often less dramatic than with the GABA agonist. As was also the case with baclofen, small concentrations of somatostatin occasionally resulted in a slowing of the spike frequency, without any overt effects on membrane potential. The effects of somatostatin persisted in TTX-treated cells but, unlike baclofen, the peptide did not appear to reduce the frequency of TTX-resistant PSP's (Fig. 2). Hyperpolarizations produced by 1 μ M somatostatin were often associated with a reduction in cell input resistance, although this was often less marked than that seen with baclofen. Responses to somatostatin showed pronounced desensitization which prevented the construction of concentration-response curves on the same cell. However, when evaluated on different cells, using the response obtained to the initial application of the peptide, hyperpolarizations to somatostatin were found to be concentration-related (Fig. 4).

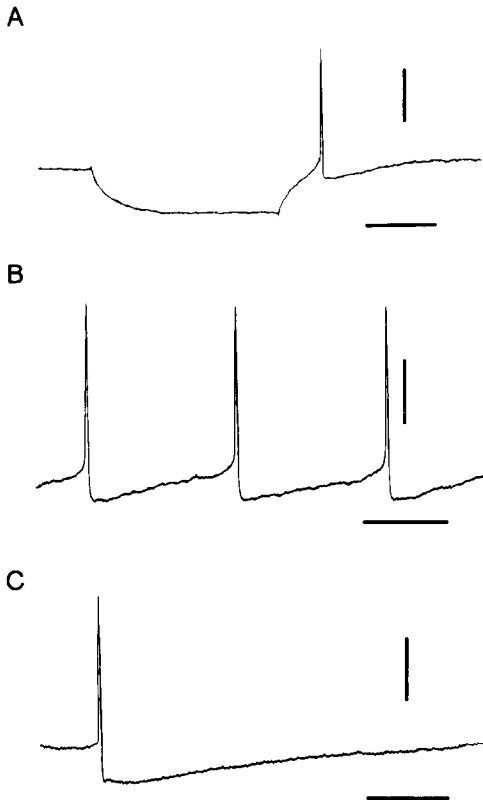


Fig. 1. Examples of the spike profiles of 3 different types of cell encountered in the present study. (A) A Type 1 neurone, which was quiescent throughout the recording period, except for the occasional anode break spike following a hyperpolarizing test pulse, as shown. (B) A Type 2 neurone, showing a relatively constant firing rate, governed by the rate of recovery from an afterhyperpolarization of medium duration. (C) A Type 3 neurone, which showed a constant rate of discharge throughout the recording period, each action potential was followed by a very lengthy biphasic afterhyperpolarization, comprizing fast and slow components. Vertical scale bars in each record represent 25 mV, the horizontal scale bars correspond to 80 msec.

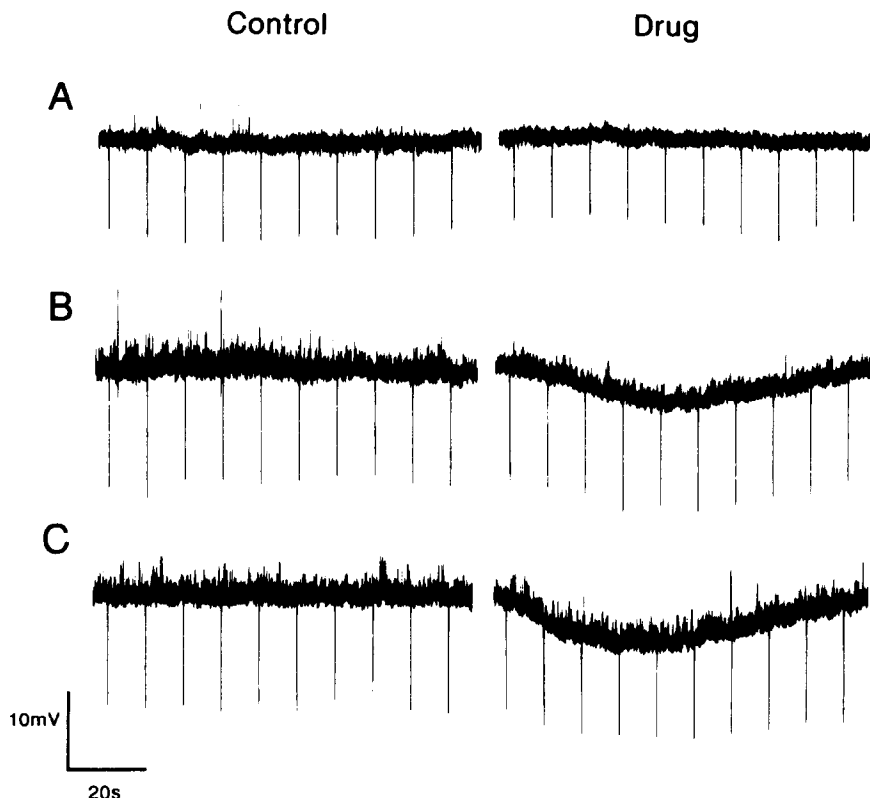


Fig. 2. Effects of bicuculline, baclofen and somatostatin on postsynaptic potentials (PSP's) recorded intracellularly from neurones of the ventromedial hypothalamic nucleus. In each record, PSP's are represented by the irregular upward deflections, arising from the baseline noise, the larger downward deflections represent the membrane potential response to electrotonic hyperpolarizing current pulses, injected at regular intervals in order to estimate membrane resistance. The figure shows periods of activity, prior to administration of drug (control) and during the perceived peak of the response to drug (drug). (A) Bicuculline ($10\ \mu\text{M}$) completely abolished PSP activity, without any effect on the membrane potential or input resistance. The PSP's returned after washing out bicuculline (not shown). (B) $(-)$ -Baclofen ($1\ \mu\text{M}$) reduced the frequency of medium and larger amplitude PSP's and hyperpolarized the membrane without, in this particular cell, any obvious effect on membrane input resistance. (C) Somatostatin ($1\ \mu\text{M}$) hyperpolarized the membrane and decreased the input resistance but did not produce any perceptible change in PSP activity. The recordings were obtained from 3 different cells, all preparations were treated with TTX ($3\ \mu\text{M}$) to abolish regenerative sodium spikes and the electrodes were filled with 3M KCl. The scale bar at the bottom of the figure applies to all records.

DISCUSSION

Cell characteristics

The present study has provided further evidence in support of neuronal heterogeneity within the ventromedial hypothalamic nucleus. In agreement with recently published results (Boden and Hill, 1988), there was strong evidence for at least two distinct populations, 1 type of cell (designated here as Type 1) being quiescent and having comparatively short membrane time constants, the remaining cells were spontaneously active, with longer time constants. This second group of cells could be further subdivided on the basis of the duration of membrane time constant which, in turn, may be an indication of differences in cell size and/or extent of dendritic arborization. The long membrane time constants were also reflected by very large input resistances for both Types 2 and 3 cells. In other respects, however,

all 3 types of cell were similar, for example, amplitude of action potentials, membrane potential and afterhyperpolarization characteristics were found not to differ significantly. The apparent differences being the result of damage from the intracellular electrode cannot be entirely ruled out, although there is supportive evidence from a previous electrophysiological study to suggest at least 3 different cell types (Minami *et al.*, 1986).

Approximately half of the cells in both Types 2 and 3 categories showed a prominent biphasic afterhyperpolarization, consisting of both fast and slow components. The remaining cells showed only the slow component. The biphasic nature of the afterhyperpolarization seen in some cells suggests that it may be comprised of at least two underlying conductances. The fact that not all Types 2 and 3 cells showed a biphasic afterhyperpolarization, may reflect cell to

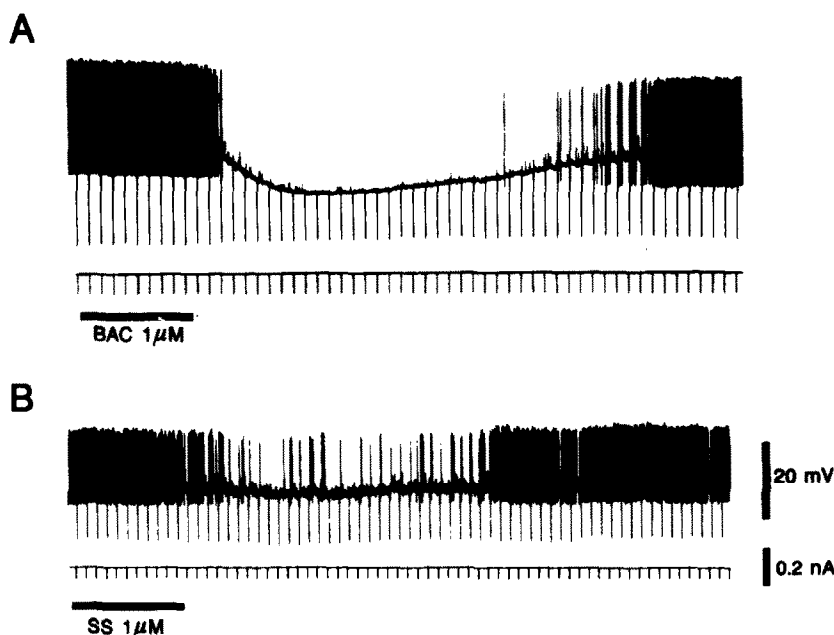


Fig. 3. Intracellular recordings from spontaneously active neurones of the ventromedial hypothalamic nucleus, showing the effects of baclofen and somatostatin. The top trace in each pair shows the membrane potential. Upward deflections are action potentials, the amplitude of which was truncated by the frequency-response characteristics of the pen recorder. The more regular downward deflections represent the response of the membrane to electrotonic hyperpolarizing current pulses, the amplitude and timing of which are shown in the lower trace in each record pair. (A) (–)-Baclofen (BAC), (B) somatostatin (SS) were applied for 1 min (horizontal bar), each agonist hyperpolarized the membrane and inhibited spontaneous action potential activity. The recording shown in (A) was from a Type 2 cell that in (B) was classified as Type 3. The response to baclofen was associated with a clear decrease in input resistance. The scale bars on the right of the figure apply to both traces.

cell variations in the degree of expression of the ion channels, underlying these conductances. It is also conceivable that the fast component may have been obscured in some neurones by the frequency-response characteristics of individual electrodes, although every effort was made to exclude such a possibility.

Pharmacological studies

Baclofen consistently hyperpolarized and inhibited the activity of neurones of the ventromedial hypothalamic nucleus, responses to the analogue of GABA were both reproducible and quantifiable. The consistency of the responses, observed between differ-

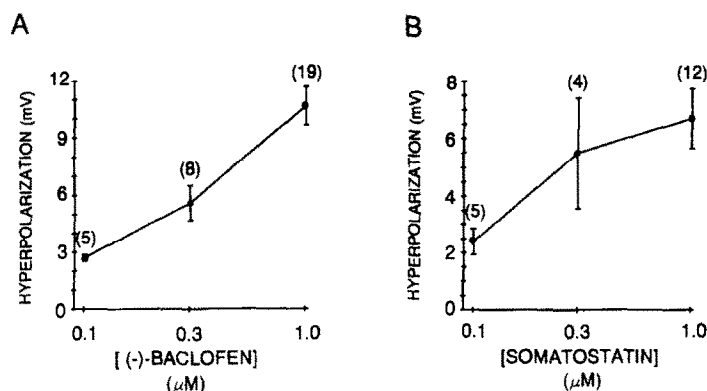


Fig. 4. Concentration-response curves for the membrane hyperpolarizing effects of baclofen and somatostatin. Mean (\pm SEM, number of observations in parentheses) amplitudes of the hyperpolarizing responses are plotted as a function of increasing concentrations of either (–)-baclofen (A) or somatostatin (B). Note that 0.1 and 0.3 μ M of the agonists produced hyperpolarizations of comparable amplitude, increasing the concentration to 1 μ M, resulted in only a small additional effect in the case of somatostatin but a significantly larger hyperpolarization in the case of baclofen.

ent cells within a single slice and between slices from different rostro-caudal loci, suggests a relatively uniform distribution of GABA_B receptors within the ventromedial hypothalamic nucleus. This may not be the case for somatostatin receptors. There was considerable cell to cell variability in the magnitude of the response to a given concentration of the peptide, particularly at larger concentrations. This may partly be the result of varying degrees of desensitization and partly due to differences in the number of receptors expressed by different cells. Variation in the density of receptors must be considered to be likely since autoradiographical studies of the hypothalamus of the rat have consistently demonstrated a comparatively sparse density of binding sites within the ventromedial hypothalamic nucleus (Uhl *et al.*, 1985; Leroux *et al.*, 1988; Krantic *et al.*, 1989). The present experiments found no evidence to suggest that the effects of either baclofen or somatostatin were mediated by specific types of cell within the ventromedial hypothalamic nucleus, since responses to each of these agonists were obtained from representatives of each of the three putative cell types.

Hyperpolarizing responses to 1 μ M of either baclofen or somatostatin were usually accompanied by a decrease in the input resistance of the cell, which persisted when the membrane potential was returned to its resting level by injection of current. The magnitude of the effect on input resistance varied considerably from cell to cell, however, in the light of previous studies with both baclofen (Gahwiler and Brown, 1985) and somatostatin (Inoue *et al.*, 1988; Pennefather, Heisler and Macdonald, 1988), it must be considered likely that this reflected an increased potassium conductance. Furthermore, in both cases this was considered to represent a postsynaptic response, since the hyperpolarizations persisted in the presence of TTX. However, presynaptic effects have also been reported for baclofen (Inoue, Matsuo and Ogata, 1985; Green and Cottrell, 1988). This may also apply in the ventromedial hypothalamic nucleus, since the frequency of observed PSP's, which reflect the spontaneous release of transmitter from presynaptic terminals, was reduced by the GABA analogue. In the case of somatostatin, the direct inhibition of voltage-dependent calcium influx (Lewis, Weight and Luini, 1986; Inoue *et al.*, 1988), suggests an additional possibility of a presynaptic effect. However, this seems unlikely in the ventromedial hypothalamic nucleus, since the peptide did not appear to affect spontaneous depolarizing PSP's.

A number of intracellular recordings appeared to indicate an inhibitory effect on spontaneous cell firing by somatostatin or baclofen, without any obvious effect on the membrane potential of the cell. Such an effect has been seen previously with somatostatin in the locus coeruleus and has been attributed to a prolongation of the spike afterhyperpolarization (Olpe, Steinmann, Pozza and Haas, 1987). Although similar effects on the afterhyperpolarization were seen

in the present experiments, it would appear that this was secondary to a membrane hyperpolarization which was small enough to be obscured by the frequency of action potentials. This was, perhaps, not surprising since the very high input resistance of Types 2 and 3 cells ventromedial hypothalamic nucleus is likely to confer an extreme sensitivity to even membrane currents of small amplitude.

In conclusion, the present *in vitro* experiments have provided further evidence that neurones within the ventromedial hypothalamic nucleus are heterogeneous and are sensitive to inhibition by drugs, acting at either GABA_B or somatostatin receptors. This is in accord with previous indications, suggesting a role for these receptors in the regulation of complex functions by the ventromedial hypothalamic nucleus. However, the effects of somatostatin and baclofen did not appear to be restricted to any electrophysiologically definable types of cell, suggesting that at least some of the diverse functions subserved by the ventromedial hypothalamic nucleus may be mediated by neurones, which are morphologically and physiologically indistinguishable.

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