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Responses to neuropeptide Y in adult hamster suprachiasmatic nucleus neurones in vitro

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Abstract

We investigated the effects of neuropeptide Y and related analogues on the extracellularly recorded spontaneous firing rate activity of adult Syrian hamster suprachiasmatic nucleus neurones in vitro. Sixty-seven neurones were tested with neuropeptide Y: 45% were suppressed, 4% were activated, and the remaining 51% were unresponsive. These responses were not blocked by the GABA receptor antagonist bicuculline, indicating that neuropeptide Y-evoked responses did not appear to be dependent on $GABA_A$ receptor activation. We tested the effects of the neuropeptide Y Y_1 receptor agonist [Leu^{31}, Pro^{34}]neuropeptide Y and the neuropeptide Y Y_2 receptor agonist neuropeptide Y-(13–36) on nine cells suppressed by neuropeptide Y in order to determine the receptor subtype(s) mediating the effects of neuropeptide Y. Four of nine cells were suppressed by [Leu^{31}, Pro^{34}]neuropeptide Y only, one of nine was suppressed by neuropeptide Y-(13–36) only, two of nine were suppressed by both compounds, while the remaining two cells did not respond to either compound. These data suggest that neuropeptide Y can modulate suprachiasmatic nucleus function directly, without recruitment of $GABA_A$ interneurones. Further, our results indicate that neuropeptide Y may act on more than one receptor subtype within the adult hamster suprachiasmatic nucleus. © 1998 Elsevier Science B.V.

Keywords: Neuropeptide Y; (Hamster); Circadian rhythm; Brain slice; GABA, (γ-aminobutyric acid); Suprachiasmatic nucleus

1. Introduction

The hypothalamic suprachiasmatic nucleus functions as the dominant circadian clock in mammals (Rusak and Zucker, 1979; Meijer and Rietveld, 1989). The suprachiasmatic biological clock is entrained to daily and seasonal variations in daylength via photic information conveyed by two visual pathways. These include a direct, monosynaptic retinal innervation of the suprachiasmatic nucleus (the retino-hypothalamic tract) and an indirect, polysynaptic pathway through retinally innervated cells of the thalamic intergeniculate leaflet which project to the suprachiasmatic nucleus (the geniculo-hypothalamic tract). Considerable evidence indicates that neuropeptide Y is an important neurotransmitter of the geniculo-hypothalamic tract. Neural tract tracing studies have shown that neuropeptide Y-containing cells of the intergeniculate leaflet project to the suprachiasmatic nucleus (Harrington et al., 1987; Takatsuji and Tohyama, 1989; Morin et al., 1992; Moore and Card, 1994). Administration of neuropeptide Y during the subjective day robustly phase-shifts the circadian rhythm in rat suprachiasmatic nucleus neuronal firing rate in vitro (Medanic and Gillette, 1993; Shibata and Moore, 1993). Similarly, microinjections of neuropeptide Y into the suprachiasmatic nucleus region at mid-subjective day also phase-resets the locomotor activity of hamsters (Albers and Ferris, 1984). The presence of neuropeptide Y binding sites in the rodent suprachiasmatic nucleus (Stopa et al., 1995) is consistent with the hypothesis that neuropeptide Y regulates circadian timekeeping within the suprachiasmatic nucleus biological clock.

Receptors for neuropeptide Y, and the structurally related peptides, peptide YY and pancreatic polypeptide, have been demonstrated in the central and peripheral nervous systems (Colmers and Bleakman, 1994). This recently expanded family of receptors includes at least six subtypes, neuropeptide Y Y_1–Y_5 and y6, based on pharmacological (agonist binding and functional potencies) and cDNA cloning studies (Gregor et al., 1996; Matsumoto et

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al., 1996; Weinberg et al., 1996; Grundemar, 1997). The neuropeptide Y Y_1 and neuropeptide Y Y_2 receptor subtypes are widely expressed in the central nervous system and have previously been characterized pharmacologically by their preferential responsiveness to [Leu^{31}, Pro^{34}]neuropeptide Y (selective for neuropeptide Y Y_1 receptors relative to neuropeptide Y Y_2 receptors) and neuropeptide Y-(13–36) (selective for neuropeptide Y Y_2 receptors relative to neuropeptide Y Y_1 receptors). Other members of this family of receptors are found in discrete nuclei of the brain, however, the neuropeptide Y receptor(s) present in the rodent suprachiasmatic nucleus are not well characterized.

Variable effects of neuropeptide Y on suprachiasmatic nucleus neurones in vitro have been previously reported. Neuropeptide Y dissolved in the medium bathing the brain slice has been found to evoke monophasic (suppressions or activations) or biphasic (activations followed by suppressions) responses on the spontaneous discharge activity of rodent suprachiasmatic nucleus neurones (Shibata and Moore, 1988; Albers et al., 1990; Liou and Albers, 1991). The possibility that these variable effects of neuropeptide Y may be attributable to the activation of different neuropeptide Y receptor subtypes has not been examined. In this study, we investigated the effects of neuropeptide Y, the neuropeptide Y Y_1 receptor agonist [Leu^{31}, Pro^{34}]neuropeptide Y, and the neuropeptide Y Y_2 receptor agonist neuropeptide Y-(13–36), on the extracellularly recorded spontaneous firing rate activity of adult Syrian hamster suprachiasmatic nucleus neurones in vitro. Further, to determine whether the effects of neuropeptide Y on suprachiasmatic nucleus neurones are mediated by activation of GABA receptors, we assessed the interactions between neuropeptide Y and the $GABA_A$ receptor antagonist bicuculline.

2. Materials and methods

Male Syrian hamsters (*Mesocricetus auratus*; 7–11 weeks of age, $n = 31$) were bred and maintained on a daily 12:12 h light/dark cycle with lights on at 0400 Greenwich Mean Time (= zeitgeber time 0). Food and water were available ad libitum. Animals were anaesthetized with halothane and decapitated during their early subjective day (zeitgeber times 5–8). Coronal slices (500 μm thick) containing the suprachiasmatic nucleus were sectioned on a Vibroslice (Campden Instruments, England) and maintained in a PDMI-2 open perfusion micro-incubator (Medical Systems, USA). Slices were mechanically stabilized within the chamber by using a customized stainless steel insert (Cutler and Mason, 1996) and equilibrated for at least 1 h prior to neurophysiological recordings. The submerged brain slices were continuously superfused with oxygenated (95% O_2: 5% CO_2) artificial cerebrospinal fluid (composition in mM: NaCl 124; KCl 3.3; KH_2PO_4 1.2; $CaCl_2$ 2.5; $MgSO_4$ 1; $NaHCO_3$ 25.5; D-glucose 10; HEPES 10; at pH 7.4) via a peristaltic pump (LKB, Sweden) at a rate of ~1.5 ml/min and maintained at

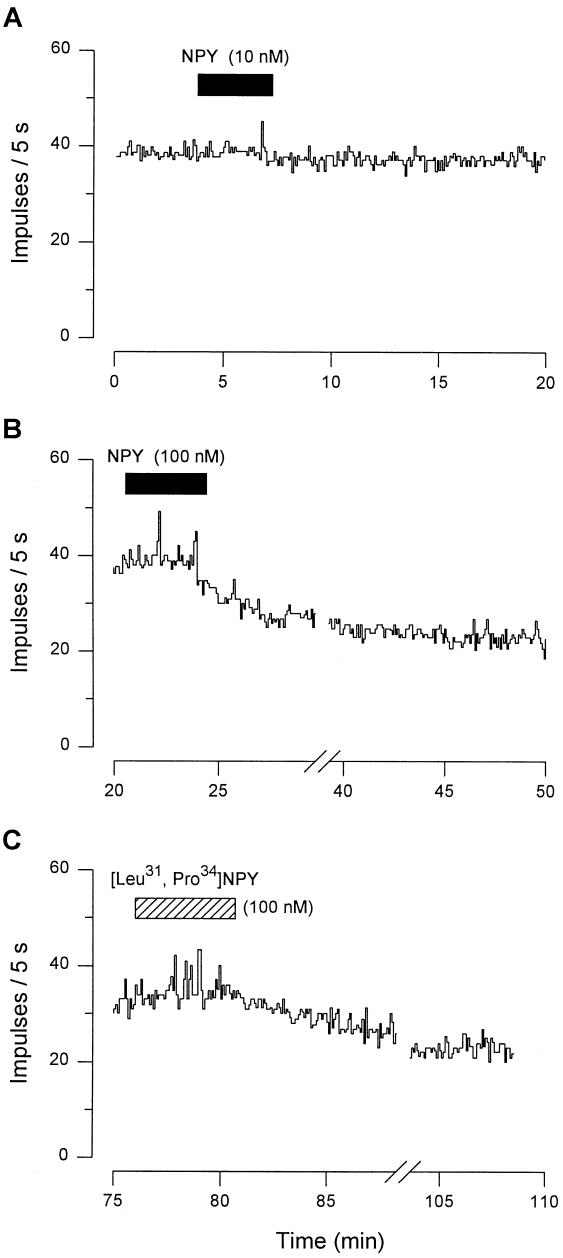

Fig. 1. Integrated firing rate histogram of a spontaneously firing neurone from the ventrolateral region of the suprachiasmatic nucleus recorded between zeitgeber times 17–19.5 for a duration of 145 min. The solid and cross-hatched bars represent the timing and duration of drug applications. (A) The record shows a low concentration of neuropeptide Y (NPY, 10 nM) eliciting a threshold reduction (5%) in cell firing rate. (B) Neuropeptide Y applied at a higher concentration produced a larger suppression in neuronal activity (35%) which had not fully recovered 50 min after neuropeptide Y treatment. (C) Application of the neuropeptide Y Y_1 receptor agonist, [Leu^{31}, Pro^{34}]neuropeptide Y ([Leu^{31}, Pro^{34}]NPY), suppressed cell firing to a similar extent (28%) which also did not fully recover after 50 min of recording. The cell was found to be unresponsive to the neuropeptide Y Y_2 receptor agonist neuropeptide Y-(13–36) (data not shown).

35 ± 0.5°C with a TC-202 bipolar temperature controller (Medical Systems, USA).

Single suprachiasmatic nucleus neurones were recorded extracellularly using glass microelectrodes filled with 2 M NaCl (resistances: 3–7 MΩ). Action potential spikes were amplified (×10 k), filtered (band-width 300 Hz–3 kHz), and displayed on an oscilloscope; single units were isolated from the background noise using a window discriminator and monitored aurally over a loud-speaker. Discriminated neuronal activity was plotted as an integrated firing rate histogram over successive 5 s epochs on a potentiometric chart recorder. Firing rate chart records were digitized off-line on a digitizing tablet using SigmaScan (Jandel, USA) software and imported into SigmaPlot (Jandel, USA) for data analysis and graphic presentation.

Human neuropeptide Y, porcine [Leu^{31}, Pro^{34}]neuropeptide Y and porcine neuropeptide Y-(13–36) were obtained from Novabiochem (England) and resuspended in 0.01 M acetic acid to stock concentration (200 μM); bicuculline methobromide (Sigma, England) was resuspended in 100% distilled water to a 10 mM stock solution. All drug stock solutions were frozen in small aliquots at −20°C. Prior to application, an aliquot was thawed and diluted in artificial cerebrospinal fluid to working concentrations; drug, or vehicle, solutions were applied to the brain slice preparation for 2–15 min via superfusion through manual switching of the perfusion line. Cells were recorded for at least 10 min until a stable baseline was obtained before evaluating any agonist-evoked responses. The criteria adopted for a responsive neurone was an agonist-evoked increase (activation) or decrease (suppression) in firing rate $>20\%$ from the basal value; mean firing rates were calculated during 3 min periods prior to agonist application (basal firing rate) and during a 3 min plateau of an agonist-evoked response. Following application of neuropeptide Y, responsive neurones were examined with the neuropeptide Y Y_1 and/or neuropeptide Y Y_2 selective agonists in random order, or the $GABA_A$ receptor antagonist bicuculline. All data are expressed as mean ± S.E.M.; statistical differences between groups of data were determined using a Student's unpaired *t*-test.

3. Results

Sixty-seven spontaneously discharging suprachiasmatic nucleus neurones were tested for responsiveness to neu-

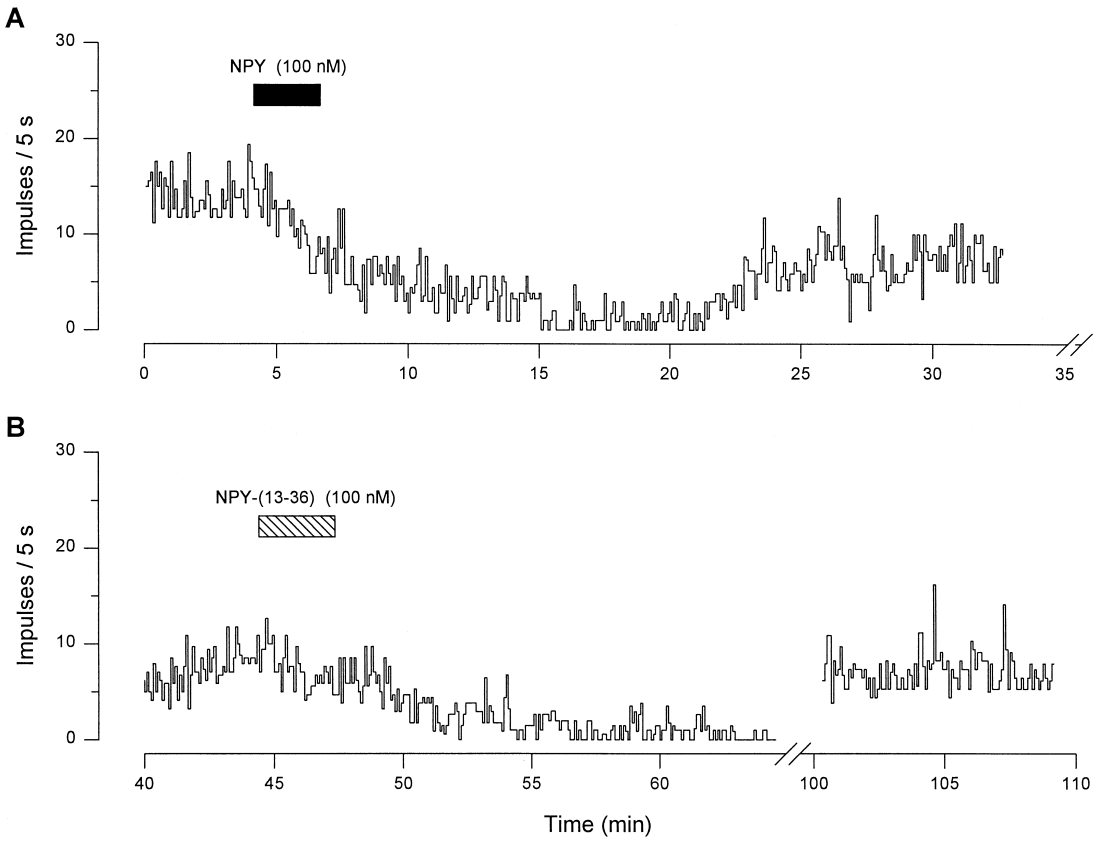

Fig. 2. Integrated firing rate histogram of a spontaneously firing neurone recorded from the ventrolateral region of the suprachiasmatic nucleus between zeitgeber times 15–18 for 170 min. The solid and cross-hatched bars represent the timing and duration of the drug applications. (A) Superfusion of neuropeptide Y (NPY) elicited a suppression of neuronal activity (93%) which only partially recovered after 40 min. (B) Application of the neuropeptide Y Y_2 receptor agonist, neuropeptide Y-(13–36) (NPY-(13–36)), also suppressed cell firing (88%) which recovered after ~60 min. The neuropeptide Y Y_1 receptor agonist, [Leu^{31}, Pro^{34}]neuropeptide Y, was ineffective on this neurone (data not shown).

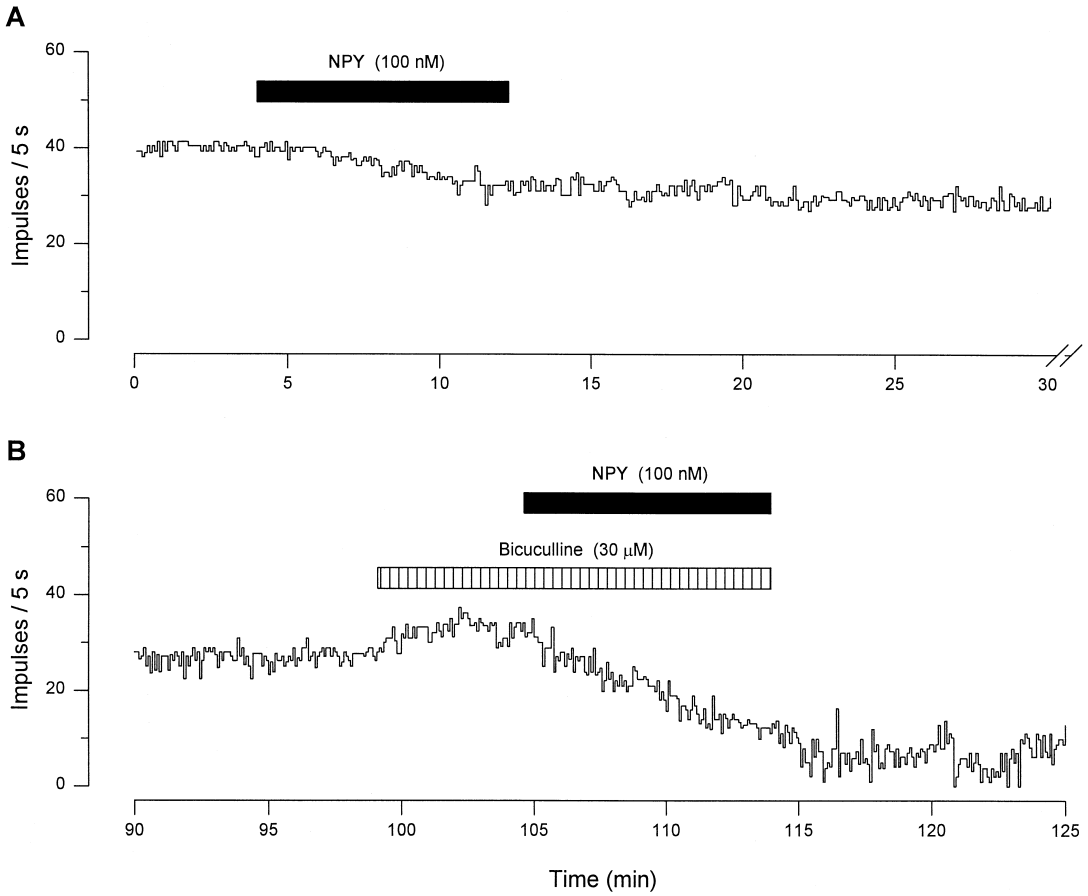

Fig. 3. Integrated firing rate histogram of a spontaneously firing neurone recorded from the ventrolateral region of the suprachiasmatic nucleus between zeitgeber times 13–15 for ~170 min. The solid and hatched bars represent the timing and duration of the drug applications. (A) A typical long-duration, neuropeptide Y (NPY)-evoked suppression in firing rate (28%) which did not return to the pre-drug baseline after ~80 min of recording. (B) Bicuculline alone elicited a 22% increase in cell firing rate; when co-applied with neuropeptide Y, bicuculline did not block the neuropeptide Y-evoked suppression in firing rate (63%), which similarly lasted for a long duration (no recovery after ~50 min of recording).

ropeptide Y (average duration of recording = 76 ± 7 min; range 11–307 min). Neuropeptide Y (10–100 nM) suppressed the firing rate of thirty cells (Figs. 1–3), activated three cells (Fig. 4), and did not elicit responses from thirty-four cells (Table 1). In contrast, vehicle applications evoked activations from two cells (Fig. 4), and no responses from the remaining twelve of fourteen cells tested (Table 1). Vehicle application followed all neuropeptide Y-evoked activations of firing rate (either an activation alone or activation followed by suppression); activational responses to neuropeptide Y were not considered to be receptor-mediated if the cell also responded to vehicle application. Two neurones which exhibited a biphasic response to neuropeptide Y (activation followed by suppression) were classified as suppressions only since subsequent application of the vehicle elicited a comparable activational response. The magnitude of the neuropeptide Y-evoked responses was similar for both the suppressions (59.1 ± 5.2%) and the activations (40.3 ± 15.4%). Marked differences in the response latency times (i.e., the time from the onset of drug application until a plateau in the response) and response recovery times (i.e., the time from drug offset until recovery to pre-drug baseline firing rate) were observed between these suppressions and activations. The response latencies for the suppressions and the activations were 6.5 ± 0.5 min and 3.7 ± 0.3 min, respectively. The average recovery time for the suppressions (41.8 ± 5.3 min) was significantly longer ($P < 0.05$) than the recovery time for the activations (4.7 ± 1.2 min). The activations may be specific to the peptide as responses to vehicle occurred immediately, in contrast to the delayed (3.7 min) activations evoked by neuropeptide Y, and lasted for the duration of the vehicle application (Table 1).

The results from all seventy-seven applications of neuropeptide Y to sixty-seven suprachiasmatic nucleus neurones revealed an apparent concentration threshold to neuropeptide Y. Concentrations of neuropeptide Y between 10–50 nM evoked responses from only two of seventeen (12%) applications (Fig. 1A), whereas 100 nM neuropeptide Y evoked responses from thirty-two of sixty (53%) applications (Figs. 1–4). Although the three neurones which were activated by neuropeptide Y were recorded from the ventrolateral portion of the suprachiasmatic nucleus, no regional differences in responsiveness to neu-

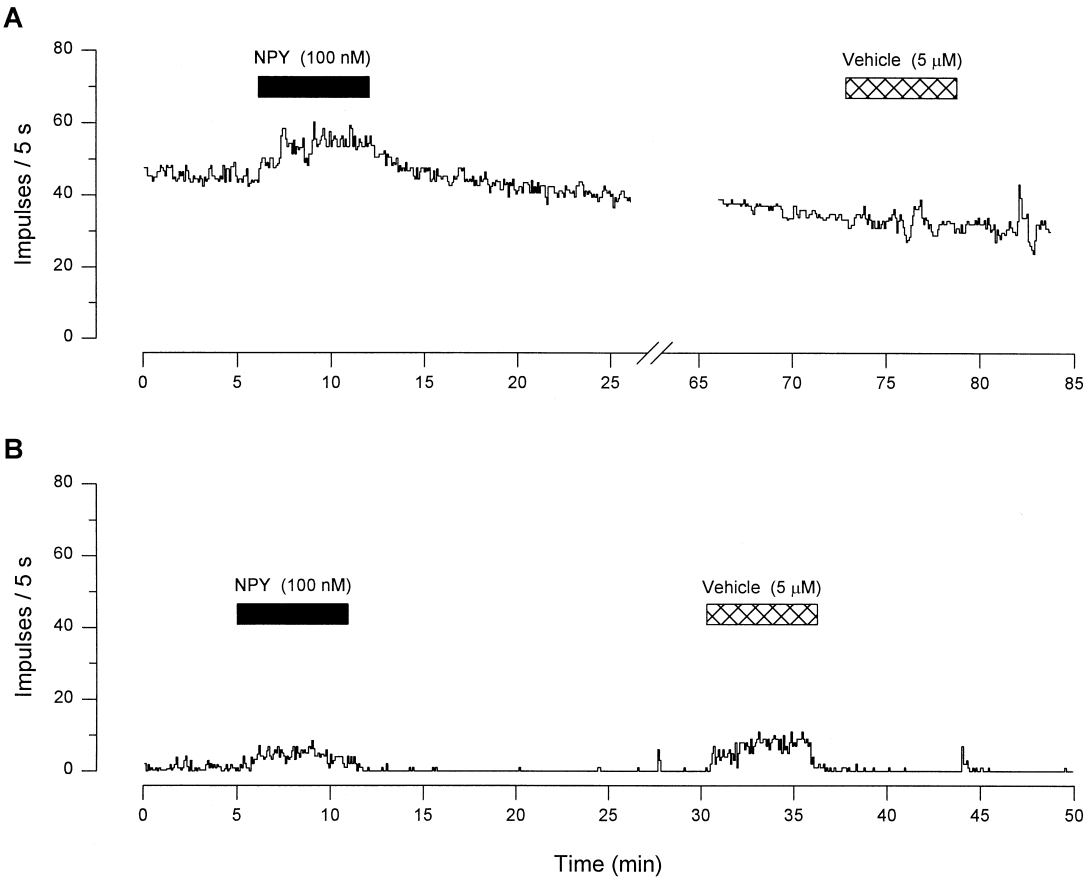

Fig. 4. Integrated firing rate histograms of two spontaneously firing suprachiasmatic nucleus neurones illustrating activations of firing rate evoked by neuropeptide Y and the specificity of these responses as assessed by vehicle control application (acetic acid at the same concentration found in 100 nM neuropeptide Y). The solid and hatched bars represent the timing and duration of the drug applications. (A) A neurone recorded from the ventrolateral portion of the suprachiasmatic nucleus between zeitgeber times 10–12 for 120 min. Neuropeptide Y (NPY) resulted in a 22% increase in firing rate which appeared to be peptide-specific since vehicle application was ineffective on cell firing rate. (B) A low firing neurone recorded from the ventrolateral portion of the suprachiasmatic nucleus between zeitgeber times 13–17 for 235 min. Application of neuropeptide Y (NPY) appeared to evoke a biphasic response, an increase followed by total suppression of cell firing rate. The neuropeptide Y-evoked activation in this instance is attributable to vehicle as subsequent acetic acid application produced a similar response. Note the characteristics (rapid onset and offset) of the response evoked by the vehicle in comparison to the response profile of the neuropeptide Y-evoked activation depicted in panel A.

ropeptide Y could be detected since similar proportions of cells in the ventrolateral (49%, $n = 20/41$) and dorsomedial (50%, $n = 13/26$) areas of the suprachiasmatic nucleus responded to neuropeptide Y. A greater proportion of suprachiasmatic nucleus cells responded to neuropeptide Y during the middle to late projected day (zeitgeber times

Table 1
Characteristics of responses evoked by neuropeptide Y, [Leu^{31}, Pro^{34}]neuropeptide Y ([Leu^{31}, Pro^{34}]NPY; neuropeptide Y Y_1 receptor-preferring agonist), neuropeptide Y-(13–36) (NPY-(13–36); neuropeptide Y Y_2 receptor-preferring agonist), or acetic acid (vehicle control) observed on hamster suprachiasmatic nucleus neuronal firing rate

Drug	Neurones tested	Activation (↑) Suppression (↓)	Responsive neurones	Response magnitude (%)	Response latency (min)	Response duration (min)
Neuropeptide Y	67	↑	3 (4.5%)	40.3 ± 15.4	3.7 ± 0.3	4.7 ± 1.2
(10–100 nM)		↓	30 (44.8%)	59.1 ± 5.2	6.5 ± 0.5	41.8 ± 5.3
[Leu^{31}, Pro^{34}]NPY	10	↑	0 (0%)	–	–	–
(100 nM)		↓	6 (60.0%)	53.5 ± 13.5	7.2 ± 1.0	42.7 ± 12.8
NPY-(13–36)	10	↑	1 (10.0%)	64.7	3.0	3.0
(100 nM)		↓	3 (30.0%)	76.7 ± 17.7	7.0 ± 0.6	39.0 ± 13.0
Acetic acid	14	↑	2 (14.3%)	110.2	1.0	1.0
(2.5–5 μM)		↓	0 (0%)	–	–	–

Data are mean ± S.E.M.

7–12; 58%, $n = 23/40$) than during the early to middle phase of the projected night (zeitgeber times 12–18; 37%, $n = 10/27$).

Ten neuropeptide Y-responsive cells (nine suppressed and one activated) were tested with both [Leu^{31}, Pro^{34}]neuropeptide Y and neuropeptide Y-(13–36) between zeitgeber times 7–19 (average duration of recording $= 149 \pm 12$ min); all agonists were used at a concentration of 100 nM. Four of the nine neuropeptide Y-suppressed neurones were also suppressed by [Leu^{31}, Pro^{34}]neuropeptide Y only (Fig. 1C), one cell was suppressed by neuropeptide Y-(13–36) only (Fig. 2B), two cells were suppressed by both agonists, and the remaining two cells were not responsive to either agonist. A single neuropeptide Y-activated neurone was activated by neuropeptide Y-(13–36) only. When compared to the neuropeptide Y-evoked response on the same cell, the responses induced by [Leu^{31}, Pro^{34}]neuropeptide Y and/or neuropeptide Y-(13–36) were directionally the same (i.e., suppressed or activated) and did not differ significantly ($P > 0.05$) with respect to response magnitude, latency, or duration (Figs. 1 and 2; Table 1).

Since GABA is believed to be a major neurotransmitter of the circadian system, and because bath application of a drug can activate transynaptic circuits which may mask the direct effects of that drug, we examined the influence of $GABA_A$ receptor blockade in five neuropeptide Y-responsive neurones recorded between zeitgeber times 8–15. Bicuculline (1–50 μM) alone increased the firing rates of all five cells tested in a concentration-dependent fashion, and the maximal concentration tested (50 μM) caused the cells to discharge in a bursting manner. However, bicuculline (10–50 μM) applied for 5–14 min prior to, and throughout the neuropeptide Y application, failed to attenuate or block either neuropeptide Y-evoked suppressions ($n = 4$; Fig. 3) or an activation ($n = 1$).

4. Discussion

These data demonstrate that the predominant response to neuropeptide Y in the hamster suprachiasmatic nucleus is suppression of the spontaneous neuronal discharge activity. These suppressions were characteristically of long duration ($\sim$39 min) and frequently did not return to pre-drug basal firing rates (Figs. 2 and 3). Similar long duration suppressions, evoked by neuropeptide Y, have been observed in the hamster (Liou and Albers, 1991) and rat suprachiasmatic nucleus (Albers et al., 1990), as well as in the rat arcuate nucleus (Rhim et al., 1997). The long duration of these responses may reflect long-term down-regulation of neuropeptide Y receptor-coupled second messenger systems (e.g., cAMP, Ca^{2+}). Such long-term suppressions (greater than one hour) of cytosolic Ca^{2+} levels induced by neuropeptide Y have been described in cultured foetal rat suprachiasmatic nucleus neurones (Obrietan and Van den Pol, 1996; Van den Pol et al., 1996). The increased proportion of suprachiasmatic nucleus cells responsive to neuropeptide Y during middle to late subjective day, when compared to early subjective night, is consistent with other reports (Mason et al., 1987; Liou and Albers, 1991) and corresponds to the phase of the circadian cycle when neuropeptide Y most effectively phase-shifts the rodent circadian clock in vitro (Medanic and Gillette, 1993; Shibata and Moore, 1993) and in vivo (Albers and Ferris, 1984). This pattern of temporal sensitivity to neuropeptide Y may represent an increased availability of neuropeptide Y receptors on suprachiasmatic nucleus neurones during this phase of the circadian cycle.

The present findings are consistent with the results of other in vitro studies where superfused neuropeptide Y predominately suppressed the firing rate of both hamster (Liou and Albers, 1991) and rat (Shibata and Moore, 1988; Albers et al., 1990) suprachiasmatic nucleus neurones. These previous studies also report neuropeptide Y-evoked activations of firing rate, which were also found in a small proportion of neurones in our study (Fig. 4). Although we observed putative biphasic responses (activation followed by suppression), and biphasic effects have previously been reported (Shibata and Moore, 1988; Albers et al., 1990; Liou and Albers, 1991; but see also Mason et al., 1987; Schmahl and Böhmer, 1997), subsequent addition of vehicle produced an equivalent activation. The activational component of any biphasic responses evoked by neuropeptide Y in the present study were attributed to the vehicle; this possibility cannot be excluded from the previously observed biphasic responses as control vehicle effects are not reported in these studies (Shibata and Moore, 1988; Albers et al., 1990; Liou and Albers, 1991).

The present findings are in contrast with those of a previous study from our laboratory where local application of neuropeptide Y evoked only activations in hamster suprachiasmatic nucleus neuronal activity (Mason et al., 1987). This disparity may be attributable to different methods of drug delivery and associated differences in local concentrations of neuropeptide Y. Pressure-ejection of neuropeptide Y through the recording micropipette (containing 50–200 μM neuropeptide Y; Mason et al., 1987) presumably results in a higher local concentration of neuropeptide to that obtained by superfusion which was used in this present study, and it is possible that the different concentrations of neuropeptide Y evoke opposite responses. We have observed such a concentration-dependent, bi-directional action of neuropeptide Y on forskolin-evoked increases in cAMP levels measured in Chinese Hamster Ovary cells expressing the cloned human neuropeptide Y_1 receptor: the forskolin response is attenuated with low (< 1 μM), while augmented with higher (≥ 1 μM) concentrations of neuropeptide Y (Selbie et al., 1997). A concentration-sensitive mechanism that differentially regulates the adenylyl cyclase system, or other neuropeptide Y receptor-coupled signal transduction pathways

(Michel, 1991), may underpin the action(s) of neuropeptide Y on suprachiasmatic nucleus neurones. Further studies are required to evaluate this possibility.

Two recent studies have found that a neuropeptide Y Y_2 agonist, neuropeptide Y-(3–36), phase-shifted both hamster behavioural rhythms in vivo (Huhman et al., 1996) and the circadian rhythm in firing rate in vitro (Golombek et al., 1996), while a neuropeptide Y Y_1 agonist ([Leu^{31}, Pro^{34}]neuropeptide Y) was ineffective in either paradigm. Our observations that suprachiasmatic nucleus neuronal firing rate can be suppressed by [Leu^{31}, Pro^{34}]neuropeptide Y and neuropeptide Y-(13–36) provides evidence that functional neuropeptide Y Y_1-like and neuropeptide Y Y_2-like receptors, respectively, are expressed in the hamster suprachiasmatic nucleus in vitro. We also observed neuropeptide Y-responsive neurones which responded to the neuropeptide Y Y_1 receptor agonist only, to the neuropeptide Y Y_2 receptor agonist only, to both agonists, or to neither agonist, which suggests a functional heterogeneity in the expression of neuropeptide Y receptors in the suprachiasmatic nucleus. These results from adult hamster suprachiasmatic nucleus neurones are consistent with those from studies on cultured foetal rat suprachiasmatic neurones (Obrietan and Van den Pol, 1996; Van den Pol et al., 1996) indicating that some neurones express both neuropeptide Y Y_1-like and neuropeptide Y Y_2-like receptor subtypes. It is possible that only the neuropeptide Y Y_2-like receptor is coupled to the phase-shifting mechanism(s) able to reset the circadian pacemaker in the suprachiasmatic nucleus; the role of the neuropeptide Y Y_1-like receptor in the suprachiasmatic nucleus is unclear at present. Further, our observations of small proportions of neuropeptide Y-responsive neurones which responded to both, or to neither neuropeptide Y receptor agonists suggest that other neuropeptide Y receptor subtypes may be present on rodent suprachiasmatic nucleus neurones. Likely candidates include the neuropeptide Y Y_3 or Y_5 receptors (Wahlestedt et al., 1991; Gerald et al., 1996) since the ligands used in this and previous studies (Huhman et al., 1996; (Golombek et al., 1996) can also bind to these neuropeptide Y receptors. Another possible mode of action is via the neuropeptide Y y6 receptor whose expression has recently been demonstrated in the mouse suprachiasmatic nucleus (Weinberg et al., 1996).

We investigated the possibility that neuropeptide Y-evoked responses might, in part, be mediated via GABA by co-applying the $GABA_A$ receptor antagonist bicuculline in the presence of neuropeptide Y. The observation of bicuculline increasing the firing rate of suprachiasmatic nucleus neurones has been previously reported (Thomson and West, 1983; Mason et al., 1991) and is presumably due to inhibition of an endogenous GABAergic tone. In the present study, bicuculline, administered at concentrations sufficient to block most GABA-mediated spontaneous outward currents (Jiang et al., 1997) or synaptic potentials (Wagner et al., 1997), was ineffective at blocking the responses evoked by neuropeptide Y (Fig. 3). These results suggest that neuropeptide Y modulation of hamster suprachiasmatic nucleus firing rate in vitro does not involve $GABA_A$ receptor activity, and are consistent with the data from other studies of rodent suprachiasmatic nucleus neurones in vitro (Obrietan and Van den Pol, 1996). Our results are also consistent with previous in vitro electrophysiological studies on the suprachiasmatic nucleus which report that neuropeptide Y-evoked suppressions of firing rate (Shibata and Moore, 1988) or phase-shifts of the circadian rhythm in firing rate (Shibata and Moore, 1993) were unaffected, respectively, in low calcium medium or by tetrodotoxin (i.e., conditions which abolish synaptic transmission). These findings suggest a post-synaptic site of action of neuropeptide Y on rodent suprachiasmatic nucleus neurones maintained in the in vitro brain slice preparation. Since the only data implicating a GABAergic modulation of neuropeptide Y-mediated effects on the suprachiasmatic nucleus originates from a study on phase-shifts of hamster behavioural rhythms (Huhman et al., 1995), it is apparent that the actions of neuropeptide Y on the suprachiasmatic nucleus may differ between in vitro and in vivo conditions. It is possible that the loss of inhibitory feedback circuitry in the slice preparation may account for the lack of effect of GABA in the mediation of neuropeptide Y effects on the circadian pacemaker contained in the suprachiasmatic nucleus. It is also possible that other neurotransmitter receptors, such as for glycine, may modulate neuropeptide Y-evoked responses in the rodent suprachiasmatic nucleus (Schmahl and Böhmer, 1997).

In summary, these data demonstrate that neuropeptide Y can modulate the spontaneous discharge activity of adult hamster suprachiasmatic nucleus neurones in a direct manner via multiple receptor subtypes. Development of more selective neuropeptide Y receptor agonists and antagonists will be required to precisely establish which neuropeptide Y receptor subtype(s) are functionally expressed in the suprachiasmatic nucleus and to determine their roles in mediating the phase-shifting actions of neuropeptide Y on the circadian pacemaker.

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