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Electrophysiological studies on rat dorsal root ganglion neurons after peripheral axotomy: Changes in responses to neuropeptides

(cholecystokinin/galanin/nerve injury/neurotensin/pain/sensory neurons)

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ABSTRACT **The effect of three peptides, galanin, sulfated cholecystokinin octapeptide, and neurotensin (NT), was studied on acutely extirpated rat dorsal root ganglia (DRGs) *in vitro* with intracellular recording techniques. Both normal and peripherally axotomized DRGs were analyzed, and recordings were made from C-type (small) and A-type (large) neurons. Galanin and sulfated cholecystokinin octapeptide, with one exception, had no effect on normal C- and A-type neurons but caused an inward current in both types of neurons after sciatic nerve cut. In normal rats, NT caused an outward current in C-type neurons and an inward current in A-type neurons. After sciatic nerve cut, NT only caused an inward current in both C- and A-type neurons. These results suggest that (*i*) normal DRG neurons express receptors on their soma for some but not all peptides studied, (*ii*) C- and A-type neurons can have different types of receptors, and (*iii*) peripheral nerve injury can change the receptor phenotype of both C- and A-type neurons and may have differential effects on these neuron types.**

Peripherally axotomized animals represent one model to study neuropathic pain (1). A damaged nerve can contribute actively to chronic pain by generating abnormal discharges and by amplifying and distorting naturally generated signals (2, 3), whereby the soma of dorsal root ganglion (DRG) neurons can become a site of abnormal impulse generation (4, 5).

Peripherally, nerve injury also causes changes in peptide expression in DRG neurons. Thus, the synthesis of substance P (6) and calcitonin gene-related peptide (7, 8) is reduced, and that of vasoactive intestinal polypeptide (9), galanin (GAL) (10), and neuropeptide tyrosine (NPY) (11) is increased. Also, the expression of neuropeptide receptors such as cholecystokinin B-type (CCK_B) receptors (12) and NPY type 1 receptors (Y1-Rs) (13) is changed. To what extent, if at all, these changes in chemical phenotype are related to pain is not well understood, but a better insight into the chemical machinery in primary sensory neurons after peripheral axotomy may provide a better understanding of pain syndromes accompanying peripheral nerve injury and could lead to improved treatment strategies.

Using electrophysiological techniques, the effects of peptides such as enkephalin (14) and GAL (15) have been studied on cultured DRG neurons. In the present study we administered three different peptides, GAL (16), sulfated CCK octapeptide (CCK-8S) (17), and neurotensin (NT) (18) onto rat DRG somata *in vitro* and monitored responses using intracellular recording techniques. Acutely extirpated DRGs from both normal rats and ganglia removed 5–7 days after peripheral axotomy were studied. We previously have reported results on the effect of NPY and NT on normal ganglia using the same intracellular recording technique (19).

MATERIALS AND METHODS

Animals and *in Vitro* Preparation. In Sprague–Dawley rats (female, 100–200 g body weight; $n = 76$), the sciatic nerve was transected bilaterally at mid-thigh level under deep anesthesia [sciatic nerve transected (SNT) rats]. After 5–7 days, these rats as well as untreated rats ($n = 89$) were anesthetized and decapitated. The lumbar (L) 4 and L5 DRGs with their dorsal roots and sciatic nerves were quickly removed from both sides of the rat. For recording, a ganglion was transferred to a submersion type chamber, through which artificial cerebrospinal fluid (ACSF) (1.5 ml/min) saturated with 95%O_2/5% CO_2 at 35–37°C was perfused. The ACSF contained, in millimolars: 124 NaCl, 2.5 KCl, 1.3 $MgSO_4$, 1.24 NaH_2PO_4, 2.4 $CaCl_2$, 25 $NaHCO_3$, and 10 glucose. The cut ends of the dorsal roots and the sciatic nerves were inserted into suction electrodes for stimulation. Conventional intracellular recordings were made using the bridge balance or discontinuous single-electrode voltage–clamp mode on an Axoclamp 2A amplifier (Axon Instruments, Foster City, CA) as described (19). For the discontinuous single-electrode voltage–clamp (switching frequencies, 4–6 kHz, duty cycle 30%), the headstage output was continuously monitored to ensure adequate electrode settling time. Neurons were routinely held near their resting membrane potential at −60 mV (holding potential). DRG neurons could be identified by their distinctive discharge and membrane properties. Some cells showed a fast conduction velocity, brief action potential (AP), and low input resistance (R_{in}), a time-dependent rectification with voltage sag during hyperpolarizing voltage transient and a strong membrane rectification when depolarized. Thus, they behaved like A-type neurons as described by Harper and Lawson (20), Todorovic and Anderson (21), and Villiére and McLachlan (22). The other cells had a slow conduction velocity, broad AP, and higher R_{in}, lacked time-dependent rectification, showed less rectification when current was injected in the depolarizing direction, and exhibited APs upon low threshold, direct somatic stimulation. They thus behaved like C-type neurons, as defined (20–22). Physiological data were accepted from neurons that had a resting membrane potential of at least −45 mV.

On acquisition of a stable recording, GAL, CCK-8S, and NT (all Bachem) were applied via the bath or pressure application through micropipette, and the changes in membrane potential or current were recorded. All data were stored on a personal

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Abbreviations: ACSF, artificial cerebrospinal fluid; AP, action potential; CCK-8S, sulfated cholecystokinin octapeptide; DRG, dorsal root ganglion; GAL, galanin; L, lumbar; NPY, neuropeptide tyrosine; NT, neurotensin; R_{in}, input resistance; SNT, sciatic nerve transected.

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Table 1. Effect of sciatic nerve cut on resting potential, input resistance, and AP waveform in small and large L4, L5 DRG neurons

	C-type		A-type	
	Normal	SNT	Normal	SNT
RP, mV	−63 ± 11.4 (n = 46)	−51 ± 15.3 (n = 30)	−60.5 ± 8.2 (n = 50)	−58.7 ± 10.3 (n = 45)
R_{in}, MΩ	98.3 ± 21.2 (n = 22)	62.4 ± 18.3 (n = 16)	33.2 ± 11.1 (n = 33)	24.3 ± 9.2 (n = 31)
AP half width, ms	2.26 ± 0.39 (n = 12)	2.53 ± 0.48 (n = 10)	1.08 ± 0.18 (n = 26)	0.61 ± 0.21* (n = 26)

Values are means ±SE. *$P < 0.05$ for comparison between normal and SNT rats. RP, resting potential; R_{in}, input resistance.

computer for on-line and off-line analysis. All data are from preparations that showed significant recovery upon washout (except where indicated differently). All data are expressed as the mean ± SEM. Statistical comparisons were performed using Student's t test, and statistical differences were considered significant at $P \leq 0.05$.

RESULTS

In total, 95 neurons were recorded from normal rats. Fifty were classified as A-type neurons, and 45 were C-type neurons (see refs. 19–21). Seventy-six neurons were recorded from SNT rats; 46 were A-type and 30 were C-type neurons. The membrane properties determined in DRG neurons from normal and SNT rats are given in Table 1. In normal rats, APs from C-type neurons were long in duration (>2 ms), displayed an inflection on the falling phase, and had a slow rate of rise. APs from A-type neurons were shorter in duration (<2 ms) and had a rapid rate of rise. After axotomy, AP from C-type neurons showed no difference in the duration. In contrast, A-type neurons had APs of a significantly shorter duration after axotomy. The R_{in} was lower in SNT rats; also, there was a difference in resting membrane potential between normal and SNT rats, but these changes were not significant. The responses of the DRG neurons to neuropeptides are summarized in Table 2.

Response to GAL. *C-type neurons.* Under voltage–clamp conditions, bath or micropipette application of GAL (0.01–1 μM) did not change the membrane current significantly in any of the 10 tested cells from normal rats but induced an inward current (amplitude range 42–91 pA) in 7 of 15 tested cells from SNT rats (Fig. 1). The current was produced ≈1–2 min after switching to the ACSF-containing GAL and over a period of 2–4 min. Within 2–6 min after switching back to control ACSF without GAL, the membrane current returned to the baseline (Fig. 1*A*). For pipette application, the latency was ≈1–2 s.

A-type neurons. Ten A-type cells from normal rats were tested with bath or micropipette application of GAL (0.01–1 μM). No significant change was observed (Fig. 1). An inward current (amplitude range 90–164 pA) was evoked in 6 of 15 tested cells from SNT rats, and the effect lasted for ≈6 min, after which recovery was observed (Fig. 1*A*). The remaining nine cells did not respond.

Response to CCK-8S. *C-type neurons.* In eight of nine tested cells from normal rats, bath or micropipette application of CCK-8S (0.01–1 μM) did not change the membrane current significantly (Fig. 2). A CCK-induced inward current could only be recorded from a single C-type neuron from normal rats. However, in SNT rats, CCK-8S induced an inward current (amplitude range 40–95 pA) in 11 of 16 tested cells. The CCK-8S-induced inward current was fully reversible and mostly lasted for 2–4 min (Fig. 1*B*).

A-type neurons. No significant change was observed in 10 A-type cells from normal rats, tested with bath or micropipette application of CCK-8S (0.01–1 μM) (Fig. 2). However, CCK-8S caused an inward current (amplitude range 78–198 pA) in 8 of 13 tested cells from SNT rats, and the duration of the effect lasted for 6–10 min (Fig. 1*B*).

Response to NT. *C-type neurons.* In normal rats, bath or micropipette application of NT (0.01–1 μM) induced an outward current (amplitude range 41–108 pA) in 7 of 26 tested cells (Fig. 3). In contrast, NT induced an inward current in 6 of 10 tested cells from SNT rats. This effect lasted for 3–6 min, and recovery was observed after a 3-to 5-min wash out with control ACSF (Fig. 1*C*).

A-type neurons. Thirty-one A-type cells from normal rats were tested with bath or micropipette application of NT (0.01–1 μM). In 14 cells, an inward current (amplitude range 95–178 pA) was evoked, and the effect was fast in onset with a duration of 6–10 min (Fig. 3). An outward current was evoked only in one A-type cell. In SNT rats, NT caused an inward current (amplitude range 84–192 pA) in 5 of 12 tested cells (Fig. 1*C*).

DISCUSSION

In this study, we have analyzed the response of DRG neurons to three neuropeptides, GAL, CCK-8S, and NT. We have compared normal L4/L5 ganglia with L4/L5 ganglia after transection of the sciatic nerve. Such a peripheral axotomy induces marked changes not only in expression of neuropeptides but also of their receptors (see ref. 23). Our results clearly indicate that receptors for these three peptides under certain conditions are functional at the level of the DRG neuron cell somata. The electrophysiological effects are in general agree-

Table 2. Effect of neuropeptides on DRG neurons from normal and SNT rats

	DRG neurons							
	Total number tested		Outward current		Inward current		No effect	
	Normal	SNT	Normal	SNT	Normal	SNT	Normal	SNT
C-type								
GAL	10	15				7	10	8
CCK-8	10	16			1	11	9	5
NT	26	15	7			8	19	7
A-type								
GAL	10	15				6	10	9
CCK-8	9	13				8	9	5
NT	31	12	1		14	5	16	7

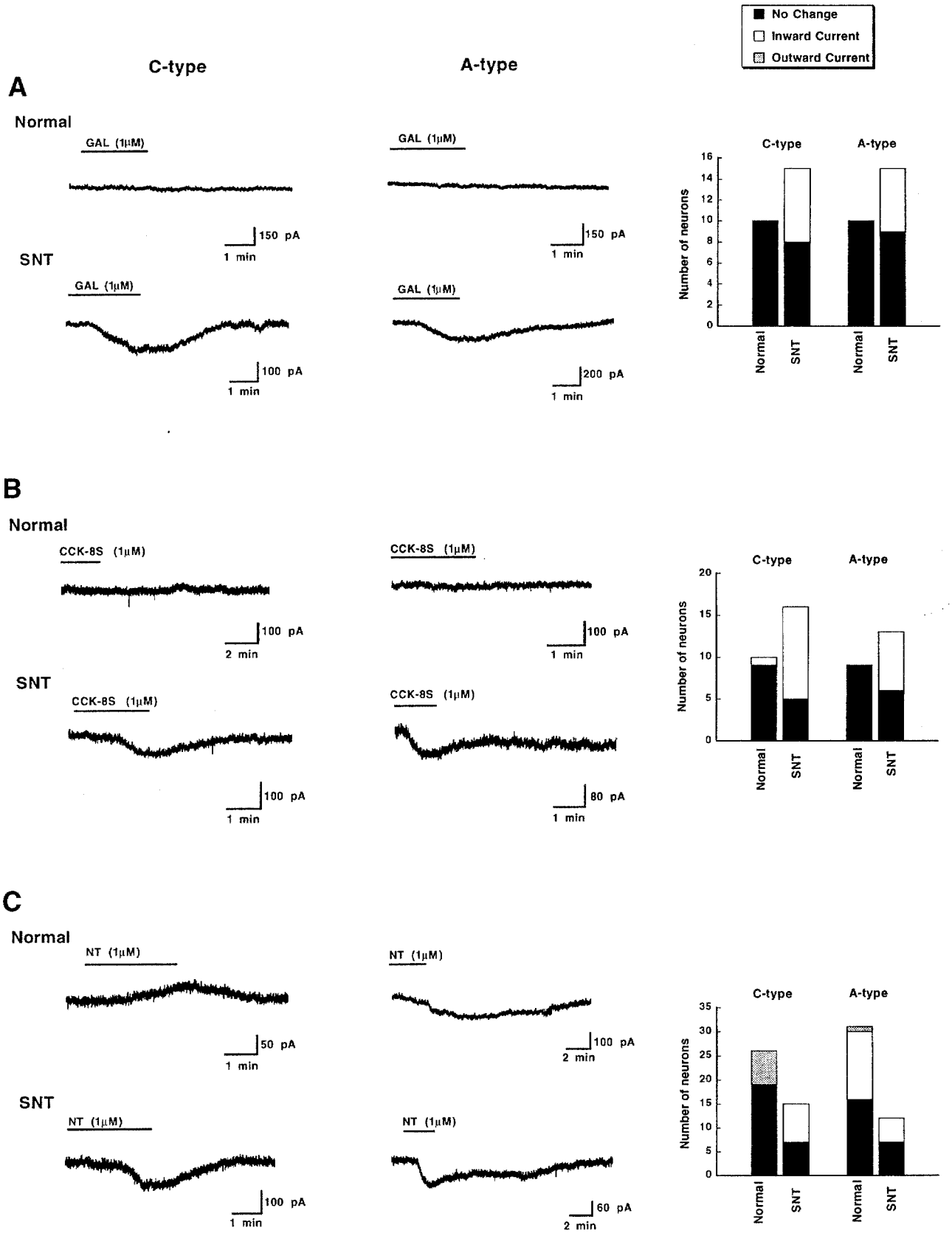

FIG. 1. (*A*) Bath application of GAL did not change membrane current in a C-type (*Upper Left*: holding potential −60 mV) or A-type (*Upper Right*: 65 mV) cell from normal rats but induced inward currents in a C-type (*Lower Left*: −60 mV) and A-type (*Lower Right*: −65 mV) cell from SNT rats. (*B*) CCK-8S at 1 μM did not change the membrane current in either a C-type (*Upper Left*: −62 mV) or A-type (*Upper Right*: −65 mV) cell from normal rats; however, it induced an inward current in both a C-type (*Lower Left*: −62 mV) and an A-type (*Lower Right*: −65 mV) cell from SNT rats. (*C*) Bath application of 1 μM NT induced an outward current in a C-type cell (*Upper Left*: −60 mV) from a normal rat and an inward current in a C-type cell from a SNT rat (*Lower Left*: −60 mV). An inward current was induced by NT in A-type cells from normal (*Upper Right*: −60 mV) and SNT (*Lower Right*: −65 mV) rats. Note the different time and current scales.

ment with the changes in receptor mRNA levels observed with *in situ* hybridization analysis of normal and axotomized DRGs, as discussed below.

We also found that the duration of APs was decreased in A-type neurons after axotomy whereas C-type neurons did not show significant changes in APs. Changes in AP shape have been reported in cultured DRG neurons extirpated from rats with sciatic nerve ligation (24) as well as in vagal parasympathetic efferent neurons after axotomy (25). However, the mechanism underlying the changes in AP shape after axotomy is unclear.

Galanin. The present electrophysiological results suggest that neither GAL-R1 (26, 27) nor GAL-R2 (28, 29) receptors normally are somatic receptors in DRGs controlling electrical membrane properties because GAL had no effect on DRG neurons taken from normal rats, despite the presence of GAL-R1 receptor mRNA (30) and GAL-R2 receptor mRNA (ref. 29; T. J. Shi, X.Z., K. Holmberg, Z.-Q.D.X., and T.H., unpublished work) in DRG neurons. After axotomy, GAL caused an inward current, which is in agreement with a study by Puttick *et al.* (15) on DRG neurons in culture. However, in view of the down-regulation of both the GAL-R1 (30) and GAL-R2 (T. J. Shi, X.Z., K. Holmberg, Z.-Q.D.X., and T.H., unpublished work) receptors in DRGs after axotomy (see also ref. 31), it seems unlikely that these receptors are involved in the depolarization described here in acutely removed ganglia or in DRG neurons in culture (15). It is therefore possible that a third GAL receptor is involved.

Several physiological studies have dealt with the effect of GAL on dorsal horn transmission. Thus, facilitation (32–34) and inhibition (35, 36) and biphasic effects have been reported (37). Electrophysiological recordings from the dorsal horn neurons show mainly inhibition (35, 38), in agreement with presence of GAL binding sites (31, 39) and GAL-R1 receptor mRNA (26, 27) in dorsal horn neurons. Taken together, physiological and histochemical studies suggest that, in normal rats, the main effect of GAL in the dorsal horn is postsynaptic (40). After axotomy, the inhibitory component of GAL on the nociceptive flexor is enhanced (41). To what extent and how the depolarizing effect of GAL on DRG somata shown here is involved is not known. It has been proposed that GAL can represent an endogenous antinociceptive messenger molecule, of importance especially after nerve injury, that is in neuropathic pain (40). This view is supported by studies with a putative GAL antagonist (42) and GAL antisense oligonucleotides (43).

Cholecystokinin. The present electrophysiological results show that very few neurons from normal DRGs responded to CCK-8S, which is in good agreement with the lack of detectable CCK_B mRNA (12) and with low CCK binding (44) in DRG neurons. This also implies that there should be no major expression of functional CCK_A receptors on DRG neuron somata in normal rats. The up-regulation of CCK_B receptors in DRG neurons after nerve injury (12) should greatly sensitize the DRG neurons, and the present electrophysiological results showing depolarization in many small and large cells after axotomy strongly suggest that the CCK_B receptor mRNA is translated into a functional receptor protein at the level of the cell somata. It has been suggested that CCK may be, functionally, an endogenous inhibitor of opioid-induced analgesia (45), which may be related to CCK in dorsal horn neurons (46–48) and to CCK_B receptors in the dorsal horn (44, 49). In agreement, Jeftinija *et al.* (50) have shown that CCK-8 excites dorsal horn neurons. It is not known if and to what extent CCK receptors in DRG neurons may be involved in the algesic effect of CCK.

NT. In a previous study, we reported that, in normal rats, an inhibitory effect of NT is observed on small (C-type) DRG neurons and that, in contrast, the major effect of NT on large (A-type) neurons is excitatory (19), findings that are confirmed here. We propose that the inhibitory effect of NT on C-type neurons is mediated via the NT-R1 receptor cloned by Tanaka *et al.* (51) because mRNA for this receptor is present in small DRG neurons, as shown with *in situ* hybridization (19). The excitatory effect on large neurons should then involve another NT receptor, which remains to be defined. However, it is possible that the same NT-R1 receptor could have two different transduction systems.

Here we observed an inward current in C-type neurons of axotomized DRGs after NT application. This is in agreement with the down-regulation of the (presumably) inhibitory NT-R1 receptor in C-type neurons (19) and suggests induction of another NT receptor in small DRG neurons, possibly the same receptor seen in large neurons of normal DRGs. In the A-type DRG neurons, NT still caused an inward current after axotomy, suggesting that this second type of NT receptor is not changed by axotomy in A-type neurons.

NT has been shown to have antinociceptive effects (52–54). These effects have been related to NT-containing dorsal horn neurons (55–57) and NT binding sites on dorsal horn neurons (58, 59). Again, a possible relation between NT receptors in DRG neurons and nociception remains to be defined.

CONCLUSION

The results suggest that peptides, potentially released either from DRG neurons and/or local dorsal horn neurons or possibly blood-borne, may influence sensory neuron functions at the level of the cell soma. It is unclear to what extent inward and outward currents in the cell somata will influence nerve impulse activity in the peripheral and central branches of the DRG neurons, in view of the considerable distance between cell body and conducting axon. Furthermore, a lack of effect on electrical membrane properties is difficult to interpret because one function of peptides may be to influence gene regulation. For example, it has been shown that NPY enhances neurite elongation (60) and that GAL promotes regeneration (61). The apparently paradoxical finding that GAL induces an inward current in DRG neurons after nerve injury when both GAL-R1 and -R2 receptor mRNA levels are down-regulated whereas no effects could be observed in normal rats when both mRNA levels are high suggests the presence of a further receptor, perhaps related to regenerative mechanisms.

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