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Neurotensin excites basal forebrain cholinergic neurons: Ionic and signal-transduction mechanisms

(electrophysiology/G protein/pertussis toxin/inward rectification/cell culture)

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ABSTRACT **The whole-cell patch-clamp technique was used to investigate the effect of neurotensin on cholinergic neurons cultured from the rat nucleus basalis of Meynert. Neurotensin excited the neurons by inducing an initial inward current carried, at least in part, by Na^+ and by reducing inwardly rectifying K^+ conductance. Reduction of the inwardly rectifying K^+ conductance was mediated by a pertussis toxin-insensitive G protein.**

Neurotensin (NT), a peptide neurotransmitter originally isolated from bovine hypothalamus (1), is widely distributed in the mammalian brain (2). NT-immunoreactive fibers innervate the nucleus basalis of Meynert (3, 4), and high-affinity NT receptors are located on the perikarya and proximal dendrites of nucleus basalis magnocellular cholinergic neurons (5). These neurons provide the major cholinergic innervation to the neocortex and amygdala (6, 7) and are thought to be involved in cortical arousal, learning, and memory. In Alzheimer disease and several other dementias, nucleus basalis cholinergic neurons degenerate (8–10).

NT excites neurons in many central nervous system regions (11–18). Excitation has been associated variously with no change or a slight decrease in membrane conductance (15), with a decrease in K^+ conductance (16), or with both a decrease in K^+ conductance and an increase in a nonselective conductance (17, 18).

We report here that NT, acting through a pertussis toxin (PTx)-insensitive G protein, produces a long-lasting excitation of nucleus basalis cholinergic neurons by reducing inwardly rectifying K^+ conductance. We also found that NT excites the neurons by inducing an additional, brief inward current that is carried, at least in part, by Na^+. A preliminary account of this work appeared in ref. 19.

MATERIALS AND METHODS

Cell Culture. Nucleus basalis neurons were cultured from 2- to 4-day postnatal Long–Evans rats (Charles River Breeding Laboratories) as reported (20), except that 2% rat serum was used, and neurons were dissociated with papain (12 units/ml). Experiments were conducted on neurons cultured for ≈2 weeks. For PTx experiments, cultures were treated as described (21). Acetylcholinesterase (AChE) staining was by the method of Karnovsky and Roots (22).

Electrophysiology. The techniques used were similar to those of Yamaguchi *et al.* (23). The whole-cell version of the patch-clamp technique (24) was used for both current-clamp and voltage-clamp recordings. The standard external solution contained 146 mM NaCl, 5 mM KCl, 2.4 mM $CaCl_2$, 1.3 mM $MgCl_2$, 5 mM Hepes·NaOH buffer, and 11 mM glucose (pH 7.4). The low external Na^+ solution contained 131 mM *N*-methyl-D-glucamine and 14.5 mM NaCl, and the high external K^+ solution contained 141 mM NaCl and 10 mM KCl, with all else as in the standard solution for both. Tetrodotoxin (0.5 μM) was added to the external solution in voltage-clamp experiments. The standard pipet solution contained 144 mM potassium D-gluconate, 8.5 mM sodium D-gluconate, 1.5 mM NaCl, 5 mM Hepes·KOH buffer, 0.5 mM EGTA·KOH, 0.25 mM $CaCl_2$, 3 mM $MgCl_2$, 2 mM Na_2ATP, 100 μM Na_3GTP, and ≈5 mM KOH (pH 7.2). When a hydrolysis-resistant GTP analogue was used, GTP was omitted. The membrane potential was corrected for the liquid-junction potential, as measured in reference to a saturated KCl electrode connected to an Ag/AgCl electrode: 14 mV between the internal and standard external solution, 21 mV for the *N*-methyl-D-glucamine external solution, and 13 mV for the high external K^+ solution. NT (1 μM unless otherwise noted), muscarine (Mus) (10 μM), and substance P (SP) (1 μM) were applied by pressure ejection from a glass pipette with a tip diameter of 3–4 μm, placed ≈40 μm from the cell soma. Bath temperature was ≈30°C. Conductance was calculated by using 50-mV hyperpolarizations from the holding potential (−79 mV). E_K was calculated from the Nernst equation. Values are given as means ± 1 SEM.

Sources of Chemicals. Chemicals used were NT (Peninsula Laboratories), guanosine 5′-[γ-thio]triphosphate (GTP[γS]) and 5′-guanylyl imidodiphosphate [Gpp(NH)p] (Sigma), PTx (List Biological Laboratories, Campbell, CA), and tetrodotoxin (Calbiochem).

RESULTS

Ionic Mechanism. We used neurons with an average soma diameter of 27 μm. Under our culture conditions such large neurons from the nucleus basalis are usually cholinergic (20). NT excited 10 of 10 such neurons from four different culture batches. In Fig. 1*A*, a brief puff application of NT depolarized and elicited repetitive spikes from a nucleus basalis neuron, which, after recording, was confirmed by histochemistry to be AChE-positive. Levey *et al.* (25) found that in basal forebrain nuclei all AChE-positive neurons are also choline acetyltransferase-positive; therefore, it is likely that AChE-positive neurons from our cultures were cholinergic. In another series of experiments, 12 of 12 nucleus basalis neurons that were excited by NT were AChE-positive (26).

Application of NT to voltage-clamped neurons revealed that two distinct ionic conductances were responsible for the excitatory effect. Membrane potential was held at −79 mV, with the K^+ equilibrium potential (E_K) at −89 mV. Membrane conductance was measured periodically by applying command voltages of +20 mV (100 msec) followed by −50 mV (100 msec). Fig. 1*B* shows a typical response in which NT

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Abbreviations: AChE, acetylcholinesterase; GTP[γS], guanosine 5′-[γ-thio]triphosphate; Gpp(NH)p, 5′-guanylyl imidodiphosphate; Mus, muscarine; NT, neurotensin; PTx, pertussis toxin; SP, substance P.

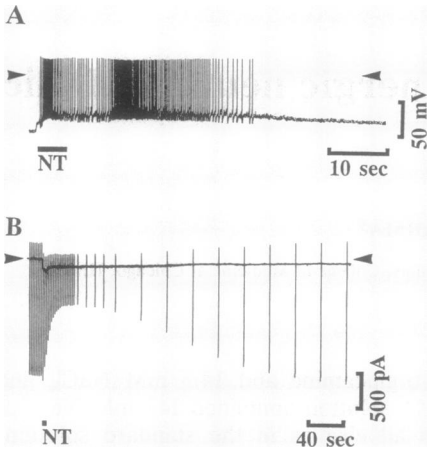

FIG. 1. NT excited nucleus basalis neurons. (*A*) NT (1 μM) was applied to a current-clamped neuron, as indicated by the horizontal bar. (*B*) Under voltage clamp, NT (0.1 μM) produced an initial inward current at the holding potential followed by a slower decline in membrane conductance. Command voltages of +20 mV (100 msec) followed by −50 mV (100 msec) were applied to monitor conductance (holding potential = −79 mV). Arrowheads indicate zero voltage (*A*) and zero current (*B*) levels.

produced a large, long-lasting decline in membrane conductance and an inward shift of the holding current. Fig. 2*A* shows that the inward shift of the holding current was composed of two partially overlapping components: an initial inward current and a long-lasting inward current. These two components can also be identified in Fig. 1*B*. The initial inward current was brief, peaking ≈5 sec after NT was applied. This current then declined, leaving the long-lasting inward current. The peak of the initial inward current (Fig. 2*A*, open arrow) occurred much before the membrane conductance was maximally reduced (Fig. 2*A*, solid arrow). On

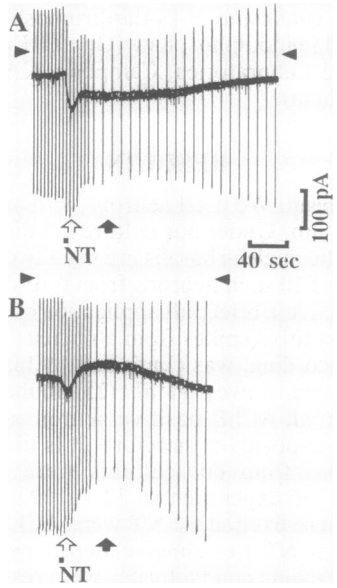

FIG. 2. NT-induced response at a holding potential more positive (−79 mV) than E_K (E_K = 89 mV) (*A*) and more negative (−99 mV) than E_K (*B*). At both potentials, NT (1 μM) produced an initial inward current (open arrows) that did not coincide with the point of maximum decrease in membrane conductance (solid arrows). In contrast, the subsequent long-lasting phase of current occurred concomitantly with the conductance decrease and reversed polarity: inward in *A* and outward in *B*. Arrowheads indicate zero voltage level. Command voltages of +20 mV (100 msec) followed by −50 mV (100 msec) were applied to monitor conductance.

average, NT elicited an initial inward current of 158 ± 22 pA that peaked after 5.2 ± 0.7 sec (n = 10) and a conductance decline of 8.2 ± 1.2 nS that peaked after 37 ± 8 sec (n = 10). If the initial inward current and the conductance decline had been two manifestations of a single ionic mechanism, their maximum points would have coincided; this did not occur, indicating that the two phenomena arose by distinct ionic mechanisms.

In Fig. 2*B*, NT was applied to a neuron held at −99 mV, 10 mV negative of E_K. NT still evoked the initial inward current, as in Figs. 1*B* and 2*A*, but the long-lasting current reversed direction from inward to outward. The current–voltage relationship of the NT-sensitive conductance was examined both soon after NT was applied, near the point of maximum initial inward current, and at a later time (>15 sec), near the point of maximum conductance decrease (Fig. 3). The NT-sensitive conductance at the later time (circles) reversed at −87.6 ± 2.1 mV (n = 8), coinciding well with E_K (−89 mV) and exhibited a rectification to the inward direction. In contrast, the reversal potential of the NT-sensitive conductance at the early phase, near the point of maximum initial inward current (squares), showed greater variability (−86 mV to −131 mV) and reversed at −104 ± 5 mV (n = 8), significantly (P = 0.02) negative of E_K. This result occurred because the reversal potential resulted from a varying mixture of initial inward current and K^+ conductance decline (27, 28).

The current–voltage relation of the NT-sensitive current was also examined with the external K^+ concentration increased to 10 mM. The current near the point of maximum conductance decrease was inwardly rectifying and reversed at −68.6 ± 0.7 mV (n = 8), close to E_K (−71 mV).

When the low external Na^+ solution was used, the initial inward current at −79 mV was reduced by ≈75%, to 39 ± 5 pA (n = 4), whereas the late conductance decrease was not diminished (9.3 ± 3.3 nS, n = 4). The current–voltage relation of the NT-sensitive current reversed at −86.7 ± 2.1 mV (n = 4), close to E_K and showed more marked inward rectification than in standard external solution (Fig. 4), as measured by the ratio of the chord conductance 20 mV depolarized versus 50 mV hyperpolarized from the reversal potential. In standard external solution the ratio was 0.56, whereas in the low Na^+ external solution it was 0.22. The more marked rectification may have occurred because unknown conductances were reduced by the low-Na^+ solution.

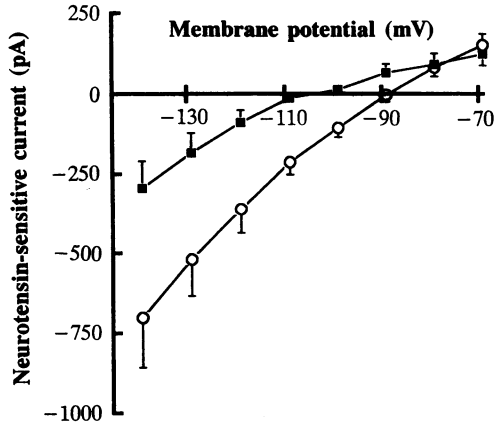

FIG. 3. Current–voltage relation of the NT-sensitive conductance. NT-sensitive current was obtained by subtracting current during the action of NT (1 μM) from current before NT application. ■, Current–voltage relation taken near the point of maximum inward current; ○, current–voltage relation taken >15 sec after NT application, near the point of maximum conductance decrease. Correction was made for series resistance. For all points n = 8; error bars indicate 1 SEM.

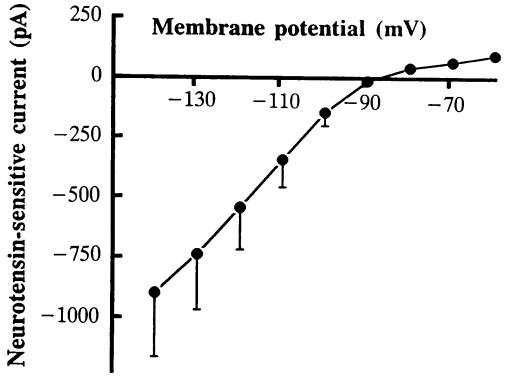

FIG. 4. Current–voltage relation of the NT-sensitive conductance in low external Na^+ solution, taken near the point of maximum conductance decrease [>15 sec after NT (1 μM) application]. Correction was made for series resistance. For all points $n = 4$; error bars indicate 1 SEM.

These results suggest that the late conductance decrease is produced by reduction of an inwardly rectifying K^+ conductance, a mechanism similar to that of the SP-induced neuronal excitation in nucleus basalis neurons (29). The behavior of the initial inward current is consistent with the idea that it is produced mainly by an increase in a nonselective ion conductance; the same mechanism was proposed to explain a similar inward current evoked by SP in locus coeruleus neurons (28, 30).

The similarity between the effects of NT and those previously observed for SP (29) on nucleus basalis neurons did not result from NT activating the SP receptor. After the SP response was desensitized by continuous bath application of SP, NT still evoked the initial inward current and decreased inwardly rectifying K^+ conductance ($n = 4$) (Fig. 5). Also, 2 of 13 neurons that responded to SP did not respond to NT.

The NT effect on the initial inward current and on the inwardly rectifying K^+ conductance had roughly the same concentration dependence. The concentration giving half the maximal response was ≈31 nM for the initial inward current and ≈24 nM for the inwardly rectifying K^+ conductance, as estimated by fitting the responses at different concentrations to a logistic equation.

Signal Transduction: G Proteins. To determine whether NT reduces inwardly rectifying K^+ conductance through a G protein, the nonhydrolyzable GTP analogues, GTP[γS] and Gpp(NH)p, were substituted for GTP in the internal solution (Fig. 6). With a nonhydrolyzable GTP analogue, a G protein-mediated response would become irreversibly activated by agonist (31). We applied NT 5 min after rupturing the patch to assure internal perfusion of the neurons with the GTP[γS]- or Gpp(NH)p-containing internal solution. During this period, no changes were seen in either conductance or holding current, indicating that little spontaneous G protein activation occurred.

Application of NT to a neuron loaded with 100 μM GTP[γS] produced the initial inward current followed by a

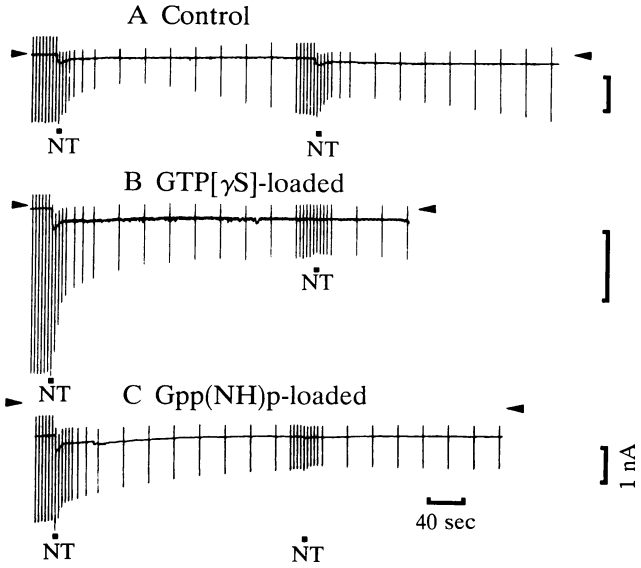

FIG. 6. NT (1 μM) irreversibly reduced inwardly rectifying K^+ conductance in nucleus basalis neurons internally perfused with nonhydrolyzable GTP analogues. (*A*) Control neuron, internal solution containing 100 μM GTP. (*B*) Internal solution containing 100 μM GTP[γS]. (*C*) Internal solution containing 1 μM Gpp(NH)p. Holding potential was −79 mV. Arrowheads indicate zero current levels. Command voltages of +20 mV (100 msec) followed by −50 mV (100 msec) were applied to monitor conductance.

decrease in membrane conductance that persisted without recovery (Fig. 6*B*) (in contrast to the recovery of conductance in the control, Fig. 6*A*). A second application of NT after 5 min produced almost no further change in either conductance or holding current. The initial inward current reached a maximum (183 ± 23 pA, $n = 8$) ≈5 sec after NT was applied, well before the membrane conductance was maximally inhibited and then declined over ≈15 sec. The remaining inward current (113 ± 22 pA, $n = 8$) then persisted almost unchanged for the duration of recording. The current–voltage relation of the conductance that was irreversibly affected by NT was examined several minutes after the peptide was applied, when both the membrane conductance and the inward current had reached nearly constant levels. The conductance was inwardly rectifying and reversed at −85.0 ± 3.2 mV ($n = 3$). In neurons loaded with 1 mM Gpp(NH)p, the results were similar to those using GTP[γS] (Fig. 6*C*).

Table 1 summarizes the effect of the nonhydrolyzable GTP analogues. In control neurons, the recovery from the NT-induced conductance decrease was complete (105%), and the second NT application produced a conductance decrease only slightly smaller than the first. In contrast, in GTP[γS] experiments, almost no recovery from the conductance decrease was seen, indicating nearly complete irreversibility, and a second NT application evoked no response. Table 1 also shows that even at a high concentration of Gpp(NH)p, the NT-induced conductance decrease recovered slightly,

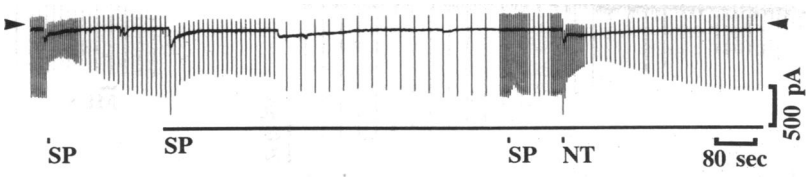

FIG. 5. NT did not act through SP receptors. A brief puff application of SP (1 μM) elicited a response similar to that evoked by NT. Continuous bath application of SP (1 μM) resulted in a response that decreased over several minutes (desensitization). A second brief puff application of SP failed to elicit a response [the small conductance decrease was an artifact (23)]. After desensitization of the SP response, the response to NT (1 μM) was normal (holding potential = −79 mV). Arrowheads indicate zero current level. Command voltages of +20 mV (100 msec) followed by −50 mV (100 msec) were applied to monitor conductance.

Table 1. Effect of GTP analogues on the NT-induced suppression of conductance

GTP analogue in patch pipette	*n*	First NT-sensitive conductance, nS	Recovery from NT effect, %	Second NT-sensitive conductance, % of first conductance
Control with GTP (100 μM)	6	7.4 ± 1.0	105 ± 16	89 ± 20
GTP[γS] (100 μM)	8	16.4 ± 1.6	1 ± 1	0 ± 0
Gpp(NH)p (1 mM)	5	13.2 ± 1.5	12 ± 9	18 ± 9

The NT-sensitive conductance was obtained by subtracting the smallest conductance seen after drug application from the conductance just before drug application. Recovery after application of NT (1 μM) was defined as the ratio X/Y, where X was given by subtracting the smallest conductance seen after NT application from the conductance immediately before the second NT application, and Y was the maximum NT-sensitive conductance during the first NT application. In GTP-perfused neurons, 105% recovery indicates that the final conductance was slightly larger than the conductance before NT application.

and a second NT application evoked a small response. This result indicates that Gpp(NH)p is not as effective as GTP[γS] in producing irreversible inhibition of the inwardly rectifying K^+ channel. The lower affinity of Gpp(NH)p for G proteins likely accounts for this result (32, 33).

Because NT irreversibly reduced inwardly rectifying K^+ conductance in the presence of nonhydrolyzable analogues of GTP, it can be concluded that a G protein mediates this effect (34). The fact that the initial inward current was not irreversibly activated under GTP[γS] or Gpp(NH)p (Fig. 6 *B* and *C*) suggests that the transduction mechanism of the initial inward current differs from that of the inwardly rectifying K^+ conductance. This result agrees with that of Koyano *et al.* (28), who observed that an initial inward current evoked by SP in locus coeruleus neurons was not irreversibly activated in the presence of GTP[γS].

PTx. Reduction of several types of K^+ currents is mediated by PTx-insensitive G proteins (21, 35, 36). Nucleus basalis cultures were treated with PTx (500 ng/ml) overnight to determine the sensitivity of the G protein mediating the NT response. PTx treatment did not abolish the effect of NT on either the initial inward current or the inwardly rectifying K^+ conductance (Fig. 7 *A* and *B* and Table 2). We confirmed the effectiveness of PTx treatment by also applying Mus to the neurons. Nucleus basalis neurons responded to Mus with an increase in conductance and an outward current (Fig. 7*C*). This Mus response was completely abolished in toxin-treated neurons (Fig. 7*D* and Table 2).

DISCUSSION

This investigation has shown that NT, acting via a PTx-insensitive G protein, reduces inwardly rectifying K^+ conductance in nucleus basalis neurons. We observed that NT elicited an additional inward current carried, at least in part, by Na^+. This current was not irreversibly activated in the presence of GTP[γS], raising the possibility that it may not be G protein-linked.

Signal Transduction. In nucleus basalis cholinergic neurons, both NT (present results) and SP (21) reduce inwardly rectifying K^+ conductance through unidentified PTx-insensitive G proteins. This result agrees with the molecular structures of the receptors, which possess the characteristics of G protein-coupled receptors (37, 38). NT (39) and SP (40, 41) stimulate inositol phospholipid hydrolysis, and the same G protein that mediates this action may also mediate inhibition of the inwardly rectifying K^+ conductance.

Ionic Mechanism. In many types of central neurons in addition to the nucleus basalis, slow excitation is mediated by the reduction of a K^+ conductance combined with the induction of a cation or nonselective conductance (17, 18, 27, 28, 30, 42). For example, in locus coeruleus neurons, SP reduces an inwardly rectifying K^+ conductance and induces a nonselective conductance (28, 30). NT also excites neurons in the rat ventral tegmental area by reducing K^+ conductance and increasing nonselective conductance (17, 18).

Nakajima *et al.* (20) previously found that SP depolarizes nucleus basalis cholinergic neurons, primarily by reducing an

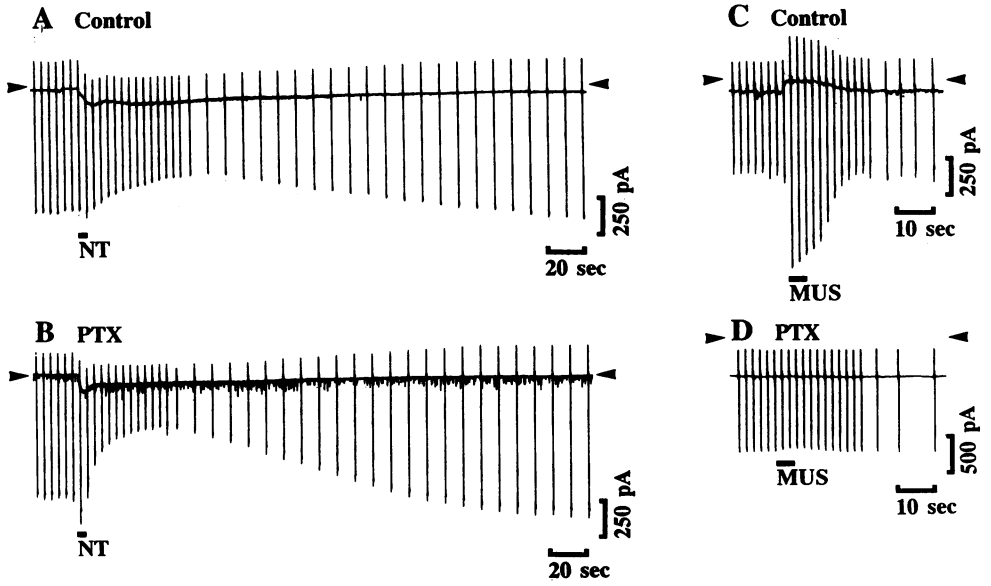

FIG. 7. PTx treatment did not significantly affect the NT response in nucleus basalis neurons. (*A*) NT (1 μM) response in a control neuron. (*B*) In a neuron treated with PTx, NT produced a response similar to the control. (*C*) Mus (10 μM) response in a control neuron. (*D*) In a neuron treated with PTx, the Mus response was abolished. Holding potential was −79 mV. Arrowheads indicate zero current levels. Command voltages of +20 mV (100 msec) followed by −50 mV (100 msec) were applied to monitor conductance.

Table 2. PTx pretreatment

Conductance	Control, nS (*n*)	PTx, nS (*n*)
Before NT	17.8 ± 2.0 (13)	21.7 ± 3.2 (13)
NT-sensitive	3.8 ± 0.6	6.4 ± 1.4
Before Mus	14.8 ± 1.8 (4)	17.2 ± 4.3 (3)
Mus-sensitive	9.8 ± 2.1	−1.2 ± 0.9

The difference in the NT (1 μM) response between control and PTx-treated neurons was not significant ($P = 0.51$), whereas the difference in the Mus (10 μM) response between control and PTx-treated neurons was very significant ($P = 0.009$). NT experiments were done on three different culture batches, and Mus experiments were done on one culture batch that responded to Mus consistently.

inwardly rectifying K^+ conductance (23, 29). However, in a small proportion of neurons SP elicited a large initial inward current (>200 pA) that occurred before the maximum decrease of K^+ conductance (21). This inward current may be the same as the initial inward current elicited by NT in the present investigation.

In vivo stimulation of the nucleus basalis leads to cortical arousal (43). The present results showed that NT consistently excited nucleus basalis neurons in culture. Also, Alonso *et al.* (44) reported that NT depolarizes basal forebrain cholinergic neurons in brain slices. NT release in the nucleus basalis may, therefore, be an important mechanism leading to cortical arousal.

It is noteworthy that the conductances affected by NT operated over different time courses. In the first few seconds after drug application, the initial inward current peaked and then declined. Thereafter, excitation continued over several minutes due to inhibition of inwardly rectifying K^+ conductance. This result may allow nucleus basalis neurons to respond quickly to NT with a short, intense burst of action potentials, followed by several minutes of slow firing and increased excitability.

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1. Carraway, R. & Leeman, S. E. (1973) *J. Biol. Chem.* **248**, 6854–6861.
2. Uhl, G. R. & Snyder, S. H. (1976) *Life Sci.* **19**, 1827–1832.
3. Jennes, L., Stumpf, W. E. & Kalivas, P. W. (1982) *J. Comp. Neurol.* **210**, 211–224.
4. Zahm, D. S. (1987) *Neurosci. Lett.* **81**, 41–47.
5. Szigethy, E., Wenk, G. L. & Beaudet, A. (1989) *Peptides* **9**, 1227–1234.
6. Wenk, H., Bigl, V. & Meyer, U. (1980) *Brain Res. Rev.* **2**, 295–316.
7. Mesulam, M.-M., Mufson, E. J., Wainer, B. H. & Levey, A. I. (1983) *Neuroscience* **10**, 1185–1201.
8. Coyle, J. T., Price, D. L. & DeLong, M. R. (1983) *Science* **219**, 1184–1190.
9. Terry, R. D. & Katzman, R. (1983) *Ann. Neurol.* **14**, 497–506.
10. Whitehouse, P. J. (1991) in *The Basal Forebrain*, eds. Napier, T. C., Kalivas, P. W. & Hanin, I. (Plenum, New York), pp. 447–452.
11. Miletic, V. & Randic, M. (1979) *Brain Res.* **169**, 600–604.
12. Andrade, R. & Aghajanian, G. K. (1981) *Soc. Neurosci. Abstr.* **7**, 573.
13. Henry, J. L. (1982) *Ann. N.Y. Acad. Sci.* **400**, 216–226.
14. Stanzione, P. & Zieglgansberger, W. (1983) *Brain Res.* **268**, 111–118.
15. Pinnock, R. D. (1985) *Brain Res.* **338**, 151–154.
16. Audinat, E., Hermel, J. M. & Crepel, F. (1989) *Exp. Brain Res.* **78**, 358–368.
17. Jiang, Z. G., Pessia, M. & North, R. A. (1992) *Soc. Neurosci. Abstr.* **18**, 272.
18. Farkas, R. H., Nakajima, S. & Nakajima, Y. (1993) *Soc. Neurosci. Abstr.* **19**, 1263.
19. Farkas, R., Grigg, J. J., Nakajima, S. & Nakajima, Y. (1992) *Soc. Neurosci. Abstr.* **18**, 1500.
20. Nakajima, Y., Nakajima, S., Obata, K., Carlson, C. G. & Yamaguchi, K. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 6325–6329.
21. Nakajima, Y., Nakajima, S. & Inoue, M. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 3643–3647.
22. Karnovsky, M. J. & Roots, L. (1964) *J. Histochem. Cytochem.* **12**, 219–221.
23. Yamaguchi, K., Nakajima, Y., Nakajima, S. & Stanfield, P. R. (1990) *J. Physiol. (London)* **426**, 499–520.
24. Hamill, O. P., Marty, A., Neher, E., Sakmann, B. & Sigworth, F. J. (1981) *Plügers Arch.* **391**, 85–100.
25. Levey, A. I., Wainer, B. H., Mufson, E. J. & Mesulam, M.-M. (1983) *Neuroscience* **9**, 9–22.
26. Farkas, R. H., Nakajima, S. & Nakajima, Y. (1994) *Neurosci. Lett.* **165**, 153–156.
27. Benson, D. M., Blizter, R. D. & Landau, E. M. (1988) *J. Physiol. (London)* **404**, 479–496.
28. Koyano, K., Velimirovic, B. M., Grigg, J. J., Nakajima, S. & Nakajima, Y. (1993) *Eur. J. Neurosci.* **5**, 1189–1197.
29. Stanfield, P. R., Nakajima, Y. & Yamaguchi, K. (1985) *Nature (London)* **315**, 498–501.
30. Shen, K.-Z. & North, R. A. (1992) *Neuroscience* **50**, 345–353.
31. Gilman, A. G. (1987) *Annu. Rev. Biochem.* **56**, 615–649.
32. Cassel, D. & Selinger, Z. (1977) *Biochem. Biophys. Res. Commun.* **77**, 868–873.
33. Bokoch, G. M., Katada, M., Northup, J. K., Ui, M. & Gilman, A. G. (1984) *J. Biol. Chem.* **259**, 3560–3567.
34. Brown, D. A. (1990) *Annu. Rev. Physiol.* **52**, 215–242.
35. Pfaffinger, P. (1988) *J. Neurosci.* **8**, 3343–3353.
36. Volterra, A. & Siegelbaum, S. A. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 7810–7814.
37. Yokota, Y., Sasai, Y., Tanaka, K., Fujiwara, T., Tsuchida, K., Shigemoto, R., Kakizuka, A., Ohkubo, H. & Nakanishi, S. (1989) *J. Biol. Chem.* **264**, 17649–17652.
38. Tanaka, K., Masu, M. & Nakanishi, S. (1990) *Neuron* **4**, 847–854.
39. Goedert, M., Pinnock, R. D., Downes, C. P., Mantyh, P. W. & Emson, P. C. (1984) *Brain Res.* **323**, 193–197.
40. Pfaffinger, P. J., Leibowitz, M. D., Subers, E. M., Nathanson, N. M., Almers, W. & Hille, B. (1988) *Neuron* **1**, 477–484.
41. Mau, S. E. & Saermark, T. (1991) *J. Endocrinol.* **130**, 63–70.
42. Murase, K., Ryu, P. D. & Randic, M. (1989) *J. Neurophysiol.* **61**, 854–865.
43. Metherate, R., Cox, C. L. & Ashe, J. H. (1992) *J. Neurosci.* **12**, 4701–4711.
44. Alonso, A., Faure, M. P. & Beaudet, A. (1992) *Soc. Neurosci. Abstr.* **18**, 761.