Substance P and NPY differentially potentiate ATP and adrenergic stimulated vasopressin and oxytocin release

JOHN R. KAPOOR AND CELIA D. SLADEK
Department of Physiology and Biophysics, Finch University of Health Sciences/
The Chicago Medical School, North Chicago, Illinois 60064

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Kapoor, John R., and Celia D. Sladek. Substance P and NPY differentially potentiate ATP and adrenergic stimulated vasopressin and oxytocin release. Am J Physiol Regulatory Integrative Comp Physiol 280: R69–R78, 2001.—The supraoptic nuclei are innervated by the A1 neurons of the caudal ventrolateral medulla. Substances colocalized in the A1 terminals include norepinephrine (NE), substance P (SP), ATP, and neuropeptide Y (NPY). ATP, acting at P2x receptors, caused rapid and sustained stimulation of vasopressin (VP) and oxytocin (OT) release from perfused explants of the hypothalamo-neurohypophyseal system. SP elicited a concentration-dependent stimulation of VP and OT release that was large and sustained compared with other stimuli. ATP, but not phenylephrine (PE, α1-adrenergic agonist), augmented the response to SP (1 μM). In contrast, NPY did not alter basal nor ATP-induced VP or OT release, but it did cause sustained potentiation of PE-induced VP and OT release. The Y1-agonist, [Leu31,Pro34]-NPY, increased VP and OT release, suggesting that the ineffectiveness of NPY reflects opposing actions at pre- and postsynaptic receptors. However, [Leu31,Pro34]-NPY did not potentiate hormone responses to ATP or PE. The differential responses to these colocalized neurotransmitters and neuropeptides illustrate the range of potential responses that stimulation of this pathway might elicit from supraoptic neurons.

nepinephrine; A1 neurons; neurohypophysis; supraoptic nucleus; hemodynamic regulation

THE MAGNOCELLULAR NEURONS of the supraoptic (SON) and paraventricular nuclei (PVN) receive a dense plexus of catecholamine-containing fibers originating from the A1 noradrenergic neurons in the ventrolateral medulla. Although abundant evidence supports the importance of the A1 pathway for stimulation of VP release in response to moderate decreases in blood pressure (9, 14), studies directed at demonstrating that norepinephrine (NE) is the primary transmitter mediating this response have not been successful (13). Recently, there has been the suggestion that coreleased neurotransmitters may be important in the regulation of hormone release. Candidates for these coreleased transmitters include the neuropeptides, substance P (SP) and neuropeptide Y (NPY), and the nucleotide, ATP. SP and NPY are localized in the A1 neurons and innervate the SON in a pattern similar to NE (3–5, 18, 39). ATP is coreleased with NE from A1 terminals in hypothalamic slices (48). Indeed, evidence for involvement of ATP in transmission by the A1 pathway exists. An antagonist of ATP receptors, suramin, blocks excitation of SON neurons by A1 stimulation or hemorrhage (6, 15). In contrast, the importance of SP and NPY in this pathway has not been evaluated.

Antidiuresis has been observed after injection of SP or senktide (a neurokinin NK3-receptor agonist) into the SON, PVN, or third ventricle (17, 36). Furthermore, intracerebroventricular injections of SP were associated with an increase in plasma VP (8). This may reflect a direct effect of SP on VP neurons as evidenced by the reports of [3H]senktide binding and the presence of NK3 receptor mRNA in the magnocellular neurons of SON and PVN (11, 16, 41). It has also been shown that the PVN and SON receive a high density of SP-like immunoreactive afferents (33), and ultrastructural evidence for SP-immunoreactive contacts on VP neurons has been obtained (21). Evidence that the majority of SP innervation of SON comes from the A1 pathway was obtained from combined immunocytochemistry/retrograde tracing studies and knife cut approaches (4). This suggests a role for SP in transmitting hemodynamic information and this is further substantiated by the report that SP content of the SON is decreased after hemorrhage (19). Despite this evidence that SP may be an important component of A1 transmission, little is known about the potential interactive capability of SP with the other coreleased transmitters from the A1 pathway.

In addition to ATP and SP, NPY and NE are colocalized in nerve terminals of the A1 pathway (5, 18, 39). Electrophysiological studies suggest that NPY might act to potentiate and prolong the excitatory effects of NE at α1-adrenoceptors on VP neurons (42, 55). To date, however, the effect of coexposure to NPY and NE or ATP on VP or OT release from the hypothalamo-neurohypophysial system (HNS) has not been evaluated.

In the present investigation, the rat HNS in vitro was used to assess the effects of SP and NPY on VP and

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OT release and their modulation of hormone responses to ATP and α-adrenergic stimulation.

MATERIALS AND METHODS

Explant preparation. Male Sprague-Dawley rats (125–149 g) were obtained from Zivic-Miller. After rats were decapitated, explants of the HNS were prepared as described previously (56). The brain and pituitary were removed from the skull using a caudal approach to maintain the pituitary stalk intact. The anterior pituitary was removed under a dissecting microscope. After gently removing the meninges (dura mater and arachnoid), a triangular block of tissue was removed from the ventral hypothalamus by cutting rostral to the optic chiasm, lateral to either side of the median eminence, and undercutting at a depth of 1–2 mm. The explants included the magnocellular neurons of the SON with their axonal projections extending through the median eminence and terminating in the neurohypophysis. With a dissecting microscope, the explants were examined to ensure that the neurohypophysial stalk was intact. Also included in the explant are the suprachiasmatic, arcuate, and ventral portions of the ventromedial, preoptic, and periventricular nuclei as well as the organum vasculosum of the lamina terminalis.

Perifusion conditions. Each explant is placed in a 500-μl perfusion chamber, maintained at 37°C in the multiple microchamber unit (Endotronics, Minneapolis, MN), and is perifused with F-12 nutrient mixture (Grand Island Biological) fortified with 20% fetal calf serum, 1 mg/ml glucose, 50 μU/ml penicillin, 50 μg/ml streptomycin, and 1 × 10^-4 M bacitracin. Bacitracin was added to the medium to prevent hormone degradation. The final osmolality of the culture medium was 295–300 mosmol/kg H2O. The medium was warmed (37°C) and gassed (95% O2-5% CO2) immediately before it entered the explant chamber. Six explants were perifused simultaneously at a rate of ~2.0 ml/h, and outflow from the chambers was collected individually in 20-min intervals using a six-place fraction collector, which was kept in a refrigerator (4°C) for subsequent measurement of VP or OT concentration. RIA was used to determine VP or OT concentration in these samples, and microvapor pressure osmometry (Wescor) was used to monitor osmolality of the perifusate.

Experimental design. Hormone release was allowed to stabilize for 4 h before exposure to any experimental conditions. During the subsequent time period, explants were perifused with basal medium or exposed to the indicated concentrations of SP (Anaspec, San Jose, CA), NPY (Anaspec), [Leu31,Pro34]-NPY (Leu-Pro; Anaspec), and/or ATP and PE (Sigma Chemical, St. Louis, MO). All drugs were dissolved directly into the medium. However, PE was added to medium containing 0.03% ascorbic acid to ensure stability. Control explants were exposed to the same concentrations of ascorbic acid.

RIA. VP and OT concentrations in the perifusate were determined by RIA as previously described (56). The antisera used were generated in conjunction with Arnel Products (Brooklyn, NY) and were used at a final dilution of 1:100,000. The buffer for both VP and OT assays was 0.1 M PBS (pH 7.6) with 1 mg/ml bovine serum albumin and 1 mg/ml sodium azide. Both assays were performed on 100- and 50-μl aliquots of each fraction collected from each explant. The standards and samples were incubated for 72 h at 4°C in the presence of 5,000 counts per minute (cpm) of 125I-labeled AVP, or 96 h at 4°C with 3,500 cpm 125I-labeled OT (New England Nuclear). Antibody-bound VP and OT were separated from free hormone with dextran-coated charcoal, and the amount of 125I-VP or OT in the pellet was determined with a gamma counter. The picograms per milliliter measurements were obtained by comparing samples to a standard curve of known concentrations of either VP or OT. All samples from a given experiment were assayed at the same time. The minimum sensitivity was 1.0 pg/tube for VP and 0.5 pg/tube for OT.

Statistical analysis. As previously mentioned, each explant was allowed to equilibrate for 4 h before exposure to drugs. Basal hormone release was calculated for each explant as the mean hormone release at the end of this equilibration period. Hormone release is expressed as a percentage of this basal value. Results are expressed as means ± SE. Statistical significance was determined on log-transformed data by ANOVA with repeated measures followed by simple main effect analysis to establish specific group differences at individual time points. Level of significance was set to P < 0.05. The probability values given in the text represent overall group comparisons from the ANOVA. The probability values on the figures represent the differences between groups at specific time points.

RESULTS

Effect of ATP on vasopressin release. As shown in Fig. 1, ATP (100 μM) caused an immediate, but unsustained, increase in VP release. ANOVA during the first hour of exposure to ATP revealed a significant increase in VP release in the ATP-treated explants (F = 10.43, P = 0.006). This concentration of ATP was selected as an effective concentration based on preliminary studies in which explants were sequentially exposed to increasing concentrations of ATP (28).

Effect of SP on VP and OT release. To evaluate the effects of SP on VP and OT release, HNS explants were
exposed sequentially to SP at increasing concentrations of 1 and 10 μM. As shown in Fig. 2, the addition of SP resulted in statistically significant, concentration-dependent increases in VP and OT release. ANOVA revealed a significant increase in VP and OT release in the SP-treated explants compared with explants maintained under basal conditions (VP: F = 14.521, P = 0.0002; OT: F = 24.126, P ≤ 0.0001). VP and OT responses to the combined exposure to SP and ATP were significantly greater

![Graph showing VP and OT release](image)

**Fig. 2.** A: VP release from HNS explants in response to increasing concentrations of substance P (SP; 1 and 10 μM). Explants were exposed to 1 or 10 μM SP beginning at the time indicated by the arrows. SP resulted in a concentration-dependent stimulation of VP release. Basal release for SP and time control groups was 81.4 ± 18.6 and 71.6 ± 11.5 pg/ml, respectively (**P = 0.000; ***P = 0.001; *P < 0.034; SP vs. time control group). B: oxytocin (OT) release from the same explants as in A. Basal release for SP and time control groups was 139.7 ± 68.6 and 116 ± 27.8 pg/ml, respectively (*P = 0.021; SP vs. time control group).

**Effect of ATP on SP-mediated VP and OT release.** To evaluate the interaction between the coreleased transmitters of the A1 pathway, ATP and SP, the less than maximally effective concentration of SP (1 μM) was used. As Fig. 3 demonstrates, VP and OT responses to 1 μM SP were augmented in the presence of 100 μM ATP. VP and OT responses to SP-ATP were greater in magnitude than the individual responses to 100 μM ATP or 1 μM SP. Furthermore, this enhanced response was sustained for the duration of the 4-h perfusion period. SP-ATP groups were run simultaneously against time control groups (basal) in one perfusion run and against groups receiving SP (1 μM) in another perfusion run. An overall ANOVA including the three groups revealed a significant difference between the groups (VP: F = 14.521, P = 0.0002; OT: F = 24.126, P ≤ 0.0001). VP and OT responses to the combined exposure to SP and ATP were significantly greater

![Graph showing VP and OT release](image)

**Fig. 3.** A: VP release in response to simultaneous exposure to ATP (100 μM) and SP (1 μM). Explants were exposed to agents beginning at the time indicated by the arrow. The combination elicits a larger and sustained response relative to the responses observed with ATP (100 μM) or SP (1 μM) individually. Basal release for SP-ATP, SP, and time control groups was 31.2 ± 4.0, 28.2 ± 18, and 27 ± 2.9 pg/ml, respectively (*P < 0.05, SP vs. SP-ATP and time control). B: OT release from same explants as in A. Basal release for SP-ATP, SP, and time control groups was 91.1 ± 61.7, 90.5 ± 55.5, and 103.6 ± 47 pg/ml (***P < 0.0001, *P < 0.034, SP vs. SP-ATP and time control).
compared with explants maintained under basal conditions (VP: $F = 27.755, P = 0.0002$; OT: $F = 34.717, P = 0.0006$) or exposed to 1 μM SP alone (VP: $F = 6.837, P = 0.0214$; OT: $F = 33.506, P = 0.0007$). Augmentation of the SP response by ATP was only apparent at the less than maximally effective concentration of SP (1 μM). When the explants were exposed to 10 μM SP along with 100 μM ATP, although the mean hormone release was somewhat higher than those exposed to 10 μM SP alone, the difference was not statistically significant (data not shown).

Effect of PE on SP-mediated VP release. To assess whether PE also altered VP responses to SP, PE (an $\alpha_1$-adrenergic agonist) was perfused along with SP (1 μM, Fig. 4). PE was used instead of NE, because NE has been shown to activate both $\alpha$- and $\beta$-adrenergic receptors with opposing actions on VP release (12). $\alpha$-Adrenergic receptors have been shown to mediate excitation of SON neurons (1). PE (100 μM) induced an increase in VP release (Fig. 5, $F = 7.522, P = 0.014$) that was similar to that observed with ATP. The peak increase (125–150% of basal) occurred slightly later than the ATP response, but it also was not sustained. PE did not cause a statistically significant increase in OT release (Fig. 5). As shown in Fig. 4, VP release was not different when explants were perfused simultaneously with SP and PE, compared with those perfused with only SP.

Effect of NPY (10$^{-5}$ M) on PE (100 μM)-mediated VP and OT release. To evaluate the effects of NPY on VP and OT release, HNS explants were exposed to NPY (10$^{-5}$ M, 10$^{-7}$ M, and 10$^{-5}$ M). There was no statistically significant difference in VP or OT release in explants exposed to NPY compared with those main-

![Fig. 4. VP release in response to simultaneous exposure to phenylephrine (PE; 100 μM) and SP (1 μM). There was no potentiation in VP release to the combined application. Explants were exposed to agents beginning at the time indicated by the arrow. Basal release for SP and SP-PE groups was 125.7 ± 78.9 and 97.2 ± 55.5 pg/ml, respectively.](image)

![Fig. 5. VP and OT release in response to the combined exposure to neuropeptide Y (NPY; 10 μM) and PE (100 μM). A: VP release was significantly increased in the NPY-PE-treated explants compared with explants exposed to PE alone (*P < 0.009). Basal release for NPY-PE, PE, NPY, and basal groups was 122.19 ± 671, 79.06 ± 39.77, 64.13 ± 48.03, and 70.29 ± 50.8 pg/ml, respectively. B: OT release in the NPY-PE-treated explants was significantly increased compared with explants exposed to PE alone (*P < 0.009). Basal release for NPY-PE and PE groups was 70.4 ± 44.88 and 77.18 ± 74.21 pg/ml, respectively, and for NPY and basal groups, which were run independently, 105 ± 16 and 82.7 ± 13 pg/ml, respectively. Explants were exposed to agents beginning at the time indicated by the arrow.](image)
NPY-PE-treated explants compared with explants exposed to 100 µM PE (VP: $F = 16.410, P = 0.0037$; OT: $F = 41.013, P = 0.0002$).

**Effect of NPY (10^{-5} \mu M) on ATP-mediated VP release.** To determine the ability of NPY (10^{-5} \mu M) to modulate the excitatory effects of ATP on VP release, HNS explants were divided into two groups. One group received ATP (100 \mu M), and the other group received the combination of ATP (100 \mu M) and NPY (10^{-5} \mu M). As Fig. 6 shows, there was no statistically significant difference in hormone release between the two groups.

**Effect of Leu-Pro (Y_1 agonist) on VP and OT release.** To assess the effect of selective activation of the NPY Y_1 receptor, explants were divided into two groups. One group was maintained under basal conditions. The other group was exposed to increasing concentrations (10^{-8} M, 10^{-7} M) of Leu-Pro at 2-h intervals. As Fig. 7A shows, Leu-Pro stimulated VP release at both concentrations. The response to the lower concentration was sustained, but in response to 10^{-7} M, VP release reached a peak and then decayed. ANOVA revealed a significant increase in VP release in the Leu-Pro-treated explants compared with explants maintained under basal conditions ($F = 32.730, P = 0.0004$). As shown in 7B, OT responses to Leu-Pro were similar to VP responses. However, ANOVA of response to both concentrations of Leu-Pro did not reveal statistically significant increases in OT release ($F = 5.341, P = 0.06$). Notice the higher degree of variability in both groups, especially at the lower concentration. ANOVA for the last 2 h (during exposure to the higher concentration) did reveal statistically significant increases in OT release ($F = 6.565, P = 0.042$), similar to the VP response.

To determine the effect of combined exposure to Leu-Pro (10^{-7} M) and PE or ATP (100 \mu M), explants were divided into two groups. One group received the Leu-Pro (10^{-7} M). The other group received the combination of the drugs. In contrast to the robust potentiation observed when NPY was perifused simultaneously with PE, there was no significant difference in VP release in the explants exposed to Leu-Pro-PE or Leu-Pro-ATP compared with Leu-Pro alone (data not shown).

**DISCUSSION**

The present studies demonstrate a role for the peptides, SP and NPY, in VP and OT release from the magnocellular neurons of the SON as well as differen-
tial interactions of these peptides with purinergic and adrenergic regulation of these neurons.

SP caused a robust stimulation of VP and OT release from HNS explants. The effect was concentration dependent and, at 10 μM SP, was large compared with other stimuli. Furthermore, the responses to both 1 and 10 μM were sustained throughout several hours of perfusion with SP. The sustained characteristic is in marked contrast to responses to other excitatory neurotransmitters such as glutamate (47, 50), ATP, and PE. These observations markedly extend the earlier report of SP stimulation of VP release from HNS explants (44), because the highest concentration of SP used in that study was 1 μM. Also, those explants were maintained in static incubation chambers. Therefore, it was not possible to appreciate the sustained nature of the response. Because both [3H]senktide binding (indicative of NK3 receptors) and NK3 receptor mRNA have been demonstrated in the magnocellular neurons of SON and PVN (11, 16, 41), the stimulation of VP and OT release by SP may reflect activation of these receptors. Activation of NK3 receptors can increase both inositol triphosphate (IP3) and cAMP, but the cAMP effect requires a 10-fold higher concentration of SP. This was demonstrated in expression studies using Chinese hamster ovary cells (37). SP increased IP3 in NK3 receptor-expressing cells at 10^{-6} M, but the cAMP response was just detectable at that concentration. Unfortunately, the effect of 10^{-5} M SP on cAMP accumulation was not evaluated, but based on the responses of the other tachykinin receptors, it is likely that this concentration would have elicited a significant increase in cAMP. Therefore, the hormonal responses to 10 μM SP that we observed might reflect activation of adenylyl cyclase as well as phosphatidylinositol (PI) hydrolysis at this concentration. Interestingly, cAMP is a potent stimulus for VP release from HNS explants (46). The thyrotropin receptor is another receptor that elicits dual activation of PI hydrolysis and cAMP cascades (52). It is possible, therefore, that depending on the concentration of the agonist, the NK3 receptors may activate more than one signaling cascade, leading to optimized hormone release. This is consistent with our finding that SP is a potent stimulus for VP release from HNS explants and also with the in vivo studies showing that SP caused antidiuresis when injected into the SON, PVN, or third ventricle (17, 36).

Although SP alone proved to be a potent stimulus for VP and OT release, due to its colocalization in the A1 neurons, we were also interested in its ability to potentiate responses to ATP and NE. To our knowledge, this is the first study to assess the interaction of SP with ATP and PE. The present data demonstrate that SP differentially interacts with these coreleased neurotransmitters from the A1 terminals. In particular, simultaneous exposure of HNS explants to ATP and SP results in an extended augmentation of both VP and OT release. The sustained nature of this augmentation demonstrates that this is more than an additive effect of exposure to two independent stimuli, because the response to ATP alone was transient. In contrast to ATP, the VP response to SP was not altered in the presence of PE. The augmentation of hormone responses by combined exposure to SP and ATP may be due to activation of converging signal transduction cascades by these agents (see Fig. 8). As discussed above, SP activation of NK3 receptors in SON may elicit PI hydrolysis and at higher concentrations, cAMP formation (37). In contrast, ATP acts on P2x ligand-gated ion channels in VP and OT neurons. This has been substantiated with intracellular recordings showing P2x receptor-mediated depolarization of VP neurons (23) and the ability of a P2x antagonist to block stimulation of hormone release from HNS explants (28). The P2x ligand-gated ion channel is permeable to Na^+, K^+, and Ca^{2+} and has the capability of transmitting fast postsynaptic potentials (7). As shown in Fig. 8, a prominent point of convergence between these signaling cascades that could lead to optimized VP and OT responses is increased intracellular Ca^{2+}. Activation of PLC by the NK3 receptor would mobilize Ca^{2+} from IP3-sensitive stores and activation of the P2x receptor by ATP would increase Ca^{2+} influx through the ATP-gated nonsclective cation channel. The internal Ca^{2+} concentration may be further augmented by ATP-mediated Na^+-dependent depolarization of the cell leading to opening of voltage-sensitive Ca^{2+} channels. In addition, because PKC is Ca^{2+} dependent, higher internal Ca^{2+} levels might contribute to an increase in the activity of PKC. Increases in PKC activity might lead to phosphorylation of the ATP-dependent P2x ion channel, leading to an increased open probability for the channel, as has been described for other channels that are phosphorylated (26, 51). Indeed, phosphorylation mediated by cAMP activation of protein kinase A might also contribute additional regulatory activation/inactivation of proteins essential for hormone release. Thus our data suggest that simultaneous activation of the P2x ligand-gated ion channel and the G protein-mediated PLC/cAMP pathway, but not redundant activation of the PLC pathway by SP and PE, potentiates VP/OT release. In vivo, the simultaneous activation of these receptors may optimize VP and OT release in response to perturbations in blood volume and/or blood pressure.

Data presented herein also demonstrate a role for NPY in the regulation of α1-adrenergic stimulation of VP and OT release from the SON. NPY (10^{-5} μM) did not alter VP or OT release from HNS explants, contrary to reports that SON injections of NPY stimulated VP release in vivo (55). This discrepancy might be explained by the marked potentiation in VP and OT release observed when NPY (10^{-5} μM) was coapplied with PE (100 μM). Previous electrophysiological data demonstrated that NPY potentiated and prolonged the excitatory effects of NE at α1-adrenoceptors on VP neurons (42, 55). Our observation of a marked potentiation in hormonal responses to NPY and PE supports the suggestion that the increase in VP release observed after SON injections of NPY in vivo reflected interactions between the injected NPY and NE being released from tonically active afferents (42). This notion might...
warrant investigation in the future by ascertaining whether coinjection of α₁-adrenergic antagonists and NPY in vivo would prevent activation of SON neurons by A1 stimulation. Interestingly, NPY-PE elicited an even greater potentiation of OT release compared with VP release.

Several mechanisms may contribute to the potentiation between NPY and PE. One possibility is that NPY prevented rapid internalization and desensitization of the α₁-adrenergic receptors. Evidence that NPY can recruit α₁-adrenergic receptors to the membrane was recently reported in kidney cells (24). Another possibility, as discussed for SP-ATP, is the convergence of intracellular signal cascades as shown in Fig. 9. NPY Y₁ receptors are best recognized as inhibitors of adenyl cyclase via coupling to a pertussis toxin (PTX)-sensitive G₁ protein. However, they have also been shown to employ G proteins to stimulate increases in inositol phosphate production, protein kinase C activity, and cytoplasmic Ca²⁺ (22, 40). Inhibition of adenyl cyclase reflects liberation of the α₁-subunit of the G₁ protein, whereas the increase in IP₃ production reflects activation of PLCB2 by the βγ complex of the G₁ protein. The mobilization of intracellular Ca²⁺ occurs from an IP₃-independent pool (40). The α₁-adrenergic receptors also activate PLC, resulting in an increase in IP₃, mobilization of IP₃-dependent Ca²⁺ stores from the endoplasmic reticulum, and activation of PKC. Thus one possible point of convergence of these two signal cascades is activation of the PLC/PKC/IP₃ cascade. Interestingly, the G₃-coupled receptors preferentially stimulate one isoform of PLCβ (β₂), whereas G₁-coupled receptors (e.g., the α₁-adrenoceptors) predominately stimulate PLCβ₁ (20). Thus convergence of these pathways leads to an increase in the PLC isoforms participating in the cellular second effector cascade, and therefore coactivation of the adrenergic and Y₁ receptors would be expected to optimize intracellular actions of the PLC enzyme. This has been observed in cells engineered to express Y₁ and α₁-AR adrenoreceptors. In these cells, agonists to the Y₁ receptor clearly potentiated the activation of PLC and PKC by PE (40). As mentioned above, in these same cells, occupation of the Y₁ receptor also elicited IP₃-independent intracellular Ca²⁺ mobilization (40). Coactivation, therefore, of the cell by PE and NPY might raise intracellular Ca²⁺ concentrations to levels that optimize the activity of Ca²⁺-dependent PKC. NPY has also been shown to potentiate PE-induced increases in PTX-sensitive cellular MAPK activity, implicating the involvement of G₁ pro-
Interestingly, NPY has been found to facilitate the interaction of the α1B-adrenoceptor with a PTX-sensitive G protein (49), representing a way by which NPY could potentiate responses to activation of the α-adrenergic receptor. Potentiation of the MAPK intracellular signaling cascade by NPY, therefore, may be another point of convergence for potentiation of physiological effects.

In addition to the above intracellular mechanisms, which might generate the synergistic response, modulation of local afferents by either NPY or PE could contribute to the synergism. For example, application of NE to hypothalamic slices increases the frequency of excitatory postsynaptic potentials in PVN magnocellular neurons (10). Similarly, inhibition of inhibitory afferents could lead to a potentiated response. Another possible site of potentiation would be modulation of stimulus-secretion coupling at the nerve terminal. NPY is colocalized in magnocellular VP neurons (32), and K+ depolarization can elicit its release from the neural lobe (31). Many of the peptides coreleased with VP and OT in the neural lobe have been shown to modulate stimulus-secretion coupling (43). However, this does not account for our results, because although NPY has been shown to potentiate K+-stimulated VP release from neural lobe, it did not alter OT release (31).

Because ATP is also colocalized with NPY in A1 terminals (5, 18, 39) and ATP can potentiate responses to PE (27), the effect of NPY on ATP-mediated hormone release was evaluated. In marked contrast to the interactive ability of NPY and PE, no potentiation in hormone responses was observed in the NPY-ATP combination. This suggests a specific mode of interaction between NPY and α1-adrenergic receptors. It also demonstrates the specificity and complexity of these signaling cascades, because clearly PLC activation and Ca2+ mobilization by Y1 receptor activation are different from PLC activation and Ca2+ mobilization in response to α1- or NK3 receptor activation in their ability to potentiate effects of ATP.

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selective $Y_1/Y_5$ agonist did not have this effect. Thus the addition of PE to NPY would replace NE inhibited by $Y_2$ stimulation and lead to enhanced VP and OT release. In contrast, Leu-Pro would only act postsynaptically on $Y_1/Y_5$ receptors, and, therefore, addition of PE would not be required to induce potentiation, because endogenous NE would not have been suppressed.

In conclusion, of the known colocalized transmitters in A1 cells (NE, ATP, NPY, and SP), as a single transmitter, SP elicits the largest and most sustained effect on VP and OT release when applied to HNS explants. This suggests that SP may play a major role either alone or in concert with coreleased ATP in the hemodynamic regulation of VP and OT release. This study also demonstrates the complex role NPY plays in the regulation of neurohypophyseal hormone release. The marked potentiation observed with combinations of SP-ATP and NPY-PE demonstrates the potential importance of coreleased transmitters for maintaining increases in both plasma VP or OT in response to activation of brain stem A1 afferents. The differential responses to neurotransmitters and neuropeptides colocalized in the A1 pathway illustrate the range of potential effects that stimulation of this pathway might elicit from SON neurons. The complexity of this regulatory input is further compounded by the likelihood that, in vivo, the release of individual neurotransmitters/neptides may vary depending on the degree to which A1 afferents are stimulated (2, 53). Thus quite variable postsynaptic responses might be achieved, reflecting the cocktail of substances present in the synapse.

Perspectives

These data demonstrate the potential importance of SP and NPY as cotransmitters in the A1 pathway mediation of hemodynamic regulation of VP release. However, these peptides may also serve other roles in the regulation of VP and OT release. Specifically, because NPY is colocalized in magnocellular VP neurons (32) and potentiates VP release from neural lobe (31), it could be part of an autostimulatory system in the VP neuron as well as serving as a transmitter for hemodynamic information. A similar autocrine role for SP in a subset of VP neurons after colchicine treatment (30). However, no mRNA for prepro-tachykinin was detected in SON under normonatremic conditions (54). SP may also serve other functions, because the perinuclear zone of SON is innervated by SP fibers (4, 30, 45). Another possibility is that additional tachykinin peptides serve as endogenous ligands for the NK3 receptors expressed in SON (16). NKB is the most potent ligand for these receptors, and several brain areas known to innervate SON or the perinuclear zone surrounding SON include cells that contain mRNA transcripts and/or immunoreactivity for NKB. These areas include the amygdala, olfactory bulb, bed nucleus of the stria terminalis, and the medial preoptic and arcuate nuclei (34, 54). Thus NKB may also serve as an endogenous ligand for the NK3 receptors in SON and may serve physiological functions other than hemodynamic regulation.

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