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NEUROMODULATORY ACTIONS OF PEPTIDES

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INTRODUCTION

Before peptides were studied thoroughly in the nervous system, they were thought of as a new category of neurotransmitters. With time, it became clear that a given neuropeptide could be involved in a variety of biological functions and that neurotransmitter-like actions alone were not enough to account for these functions. At the same time, it was found that many peptide actions had slow time courses (1–3); that there were mismatches between locations of peptides and their receptors in the brain (4); and that many neuropeptides coexisted with other transmitter agents in individual neurons (5, 6). Assuming that "neuromodulation" includes a slow time course of action, a more diffuse site of action, and an ability to alter responses to transmitters (7), these findings strongly suggested that neuropeptides can serve as neuromodulators as well as neurotransmitters. Indeed, peptide neuromodulatory actions have been demonstrated by numerous studies. In the present review, we examine the characteristics, explore underlying mechanisms, and assess the biological and pharmacological significance of peptide neuromodulation.

Because the term "neuromodulation" has been used in various ways, it is necessary to define the term here in order to limit the scope of the review (see also Refs. 8, 9). In neuromodulation, as reviewed here (as distinguished from a synergistic or additive action), the modulator itself has no direct effect on the substrate or has an effect that is independent of the modulation. The substrate whose response is measured can be a subcellular organelle, a nerve, endocrine or muscle cell, a neural circuit, a transmitter system, or an organ system. The effector whose direct action on the substrate

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is modulated can be a neurotransmitter, a peptide, or an electrical stimulation. Accordingly, a response can be a change in transmembrane ion fluxes, neuronal activities, transmitter or hormone release, reflexes, or behaviors. Because of limited space and because most of the peptides studied in invertebrates are not the peptides of major interest in vertebrates, this review is confined to neuromodulation observed in vertebrates.

GENERAL CHARACTERISTICS OF PEPTIDE NEUROMODULATION

Scope

The entire scope of peptide modulation is not yet strictly definable, because the field is expanding rapidly and, in particular, some peptides are studied intensively and have been shown to have many modulatory actions while others are still poorly investigated. Nevertheless, from what already has been published, it is obvious that the scope of peptide neuromodulation is extensive.

IN TERMS OF THE VARIETY OF PEPTIDES A survey of the literature shows that almost every peptide studied exhibits some kind of modulatory action. Thus, the list of peptides capable of modulation is long and includes at least the following: adrenocorticotropin (ACTH) (10), angiotensin II (AII) (11), bombesin (BBS) (12), calcitonin-gene related peptide (CGRP) (13), cholecystokinin (CCK) (11, 14–17), corticotropin releasing factor (CRF) (18), FMRFamide (19), galanin (20, 21), insulin (22, 23), luteinizing hormone releasing hormone (LHRH) (24–26), melanin inhibiting factor (MIF) (cf 27), neuropeptide Y (NPY) (28–30), neurotensin (NT) (31), opioids (32–34), oxytocin (OXY) (35), prolactin (PRL) (36–38), somatostatin (SST) (39, 40), substance P (SP) (11, 41, 42), thyrotropin releasing hormone (TRH) (43–48), vasoactive intestinal peptide (VIP) (49, 50), arginine vasopressin (AVP) (35, 51, 52). As neuromodulation draws more attention, this already impressive list will surely grow longer.

IN TERMS OF SUBSTRATES In addition to modulating neurons in the central nervous system, neuropeptides may also modulate the responses or functions of a wide variety of other types of cells, including neurons in the autonomic nervous system (24, 42, 49, 50, 53–57) and the peripheral nervous system (58–60); cardiac (61–64), smooth (65), and skeletal muscles (60); endocrine cells (66–69); and glial cells (70).

IN TERMS OF THE VARIETY OF REPONSES MODULATED Peptides can modulate biological events that range from responses by subcellular components to behaviors of the whole animal. At subcellular levels, evidence is

strong that peptides can modulate stimulant-evoked ion flux through the membrane (59, 62) and enzyme activity (71). At a cellular level, it is well-known that peptides can modulate effector-induced release of transmitters (58, 69, 72) or hormones (20, 33) and the changes in single neuron activity evoked by classical neurotransmitters (39, 43, 73), by neuropeptides (19), and even by nontransmitter agents such as glucose (23). Also known to be modulated are spinal reflexes (13, 74–77); system reactions such as intestinal secretion (78), blood pressure (29, 79, 80), body temperature (81, 82), and pseudopregnancy (25); and behaviors induced by one means or another (19, 22, 31, 83–85). Modulations at different biological levels may be causally related. For instance, attenuation of K^+-induced SP release from cultured sensory neurons by enkephalin (ENK) may be due to ENK attenuation of Ca^{2+} influx (59). However, in most instances we do not yet know the manner in which subcellular and cellular changes form the mechanisms for neuromodulations studied at the system or behavioral level.

Specificity

The phenomenon of peptide neuromodulation has been observed by so many investigators with so many peptides that it could not be an experimental artifact. However, its extensive scope, together with the fact that modulation is "indirect" in the sense of requiring the action of another agent, does raise questions of specificity. Therefore, most investigators dealing with peptide modulation have made at least some effort to assess the specificity of the neuromodulation in question. As is illustrated below, such efforts have shown that peptide neuromodulation can be specific in all aspects examined.

PEPTIDE-SPECIFIC Since many peptides can modulate a given type of response (see section on convergence), it has been a common practice for investigators to evaluate specificity by testing whether modulation by one peptide can be duplicated with other, unrelated peptides. With rare exceptions, peptide-specificity has been demonstrated. For example, we have tested preoptic neurons whose responses to norepinephrine (NE) were modulated by LHRH, with TRH as well, and have found that none of the neurons modulated by LHRH were also modulated by TRH (26). This specificity was demonstrated in spite of the fact that TRH itself could modulate NE-responses in the ventromedial hypothalamus (43). Similar specificity has been observed in cardiac muscle, where the NE-induced chronotropic response was modulated by ENK but not by β-endorphin (β-END) (62). In addition, such peptide specificities have been reported for the modulation of neuronal responses to transmitters (32, 53); of stimulus-evoked transmitter release (72, 86) or peptide releases (59, 87); of responses from cardiac muscle (61); of reproductive function (25); and of behavior (88). In one exceptional case, two

unrelated peptides, SP and SST, were found to have similar modulatory actions on the stimulus-evoked release of catecholamines (69, 70); and, in another case, both cholecystokinin octapeptide (CCK-8) and SST were seen to modulate evoked release of acetylcholine (72). However, in the latter case, at least, different peptides modulated the same reponse through distinctively different mechanisms, and each mechanism was peptide-specific (72). Therefore, even in these exceptional cases the principle of peptide-specificity might not have been violated.

To evaluate the role of peptide structure in the generation of specificity, closely related peptides have been surveyed. In one case, it was found that only ACTH and none of its fragments, ACTH1–3, 4–10, 1–16, 5–16, or 11–24 could potentiate the cardiac ionotropic response to NE (61). In our laboratory, LHRH was compared with analogues in which the primary structure was modified but the amphiphilic secondary structure was maintained. We found that the modulatory actions of LHRH could be duplicated by its amphiphilic analogues (73). It is interesting to note that in both the cerebral cortex (45) and the hypothalamus (43), the modulatory actions of TRH could be produced by its metabolite, cyclo[histydyl-prolyl] (cHP), which has some resemblance in primary, but not secondary, structure to TRH.

SITE SPECIFIC Some studies have compared the effects of a peptide on neurons located in different brain regions but showing the same response to a given effector. For example, neurons in both lateral and medial septum can be excited by glutamate, but essentially only the neurons in the lateral septum were potentiated by AVP and OXY (35). Similarly, in the cortex and caudate, but not in the hippocampus, AVP can inhibit NE-induced synthesis of cyclic AMP (cAMP) (89). The inhibitions of K^+-induced ACh release by CCK-8 and SST (72) and glutamate release by SST (90) were observed in the caudate nucleus but not in the cerebral cortex. In the periphery, NPY can potentiate NE-induced vasoconstriction, but only in muscular arteries and not in veins or aorta (30). At the behavioral level, it has been shown that the inhibitory effect of dopamine in nucleus accumbens but not in the caudate nucleus was potentiated by CCK-8 (88).

EFFECTOR AND RESPONSE SPECIFICITIES In the cases where a response can be evoked by more than one effector, a neuropeptide modulation generally only affects the response to a specific effector, be it a neurotransmitter (10, 32, 43–45, 47, 49, 50, 53, 55, 69, 70), a nontransmitter agent (81, 91), or stimulation (40, 76). This can be illustrated with responses evoked by both nicotinic and muscarinic agonists (nACh and mACh, respectively). In the induction of membrane current (53) and the release of NE (69) the action of nACh, but not that of mACh, was modulated by SP, whereas in the evocation

of neuronal excitation (49, 50, 55) the action of mACh, rather than that of nACh, was modulated by VIP. Other examples showed that ACTH attenuated inhibitory responses of hippocampal neurons to NE but not those to serotonin (5HT) (10); in modulating the responses of neurons in the visual cortex, SST affected only those evoked by visual stimulation with preferred direction and had no effect on those with nonpreferred direction (40); low concentration of SST potentiated the flexion reflex evoked by thermal but not that by mechanical stimulation (76).

Response specificity has also been investigated and has shown where a given effector can evoke more than one response. For example, SST attenuated, in one case, only glutamate release and not the release of other amino acids from the striatum in response to high K^+ concentration (90), and, in another case, only the release of NE and not dopamine (DA) or 5HT from the hypothalamus in response to stimulation (92).

In view of the various dimensions of neuromodulatory specificities, it is obvious that differences in sites of observation, effectors used, or in responses monitored can make a big difference in determining whether and how a peptide modulates. For example, in the cerebral cortex, TRH was reported to modulate the action of glutamate, but not aspartate, agonists (45), while in motoneurons both glutamate and aspartate responses were potentiated by TRH (48). Thus, one has to exercise caution in comparing peptide modulations observed in different studies.

Relation of Neuromodulation to Colocalization with Transmitters

Through the use of immunohistochemical techniques, the coexistence of peptides with classical transmitters or other peptides within individual neurons has been shown to be more the rule than the exception (6, 93, 94), and interactions between peptides and the coexisting transmitters often have been demonstrated (5, 6). In many situations of colocalization, modulation by neuropeptides of the action of coexisting transmitters has been observed. One such system is the neurons in the ventral tegmental area that contain both CCK and DA (CCK/DA neurons) and that project to the caudal, medial nucleus accumbens, amygdala, and olfactory tubercle (94, 95). Functional studies showed that, in these brain regions, CCK can attenuate K^+-evoked DA release (96), increase the number of DA binding sites (97, 98), attenuate DA action in activating adenylate cyclase (99), potentiate the inhibitory action of DA and apomorphine (14–16), and potentiate DA- and apomorphine-induced behaviors (88). Similar relationships between peptides and coexisting neurotransmitters have also been shown for the potentiation by SP and the attenuation by CRF of seizure behavior induced by ACh in the medial frontal cortex (18), the modulation of ACh action by VIP in autonomic ganglia (49,

50), and the attenuation of ACh-evoked catecholamine release from chromaffin cells by an opioid peptide, met-ENK[Arg^{6}Phe^7], which appears to coexist with ACh in the splanchnic axons (67). Neuromodulation can also occur between colocalized peptides. The potentiation and prolongation of SP action by CGRP in the modulation of the flexion reflex (100) is an example.

Coexisting neuropeptides and neurotransmitters do not always interact by modulation: many of them interact by "cooperation" or synergism (5, 6). In some cases, no interaction has been found. The effects of SST and GABA in neurons of the visual cortex may be such a case. As reviewed by Sillito (40), although the two substances evidently coexist in certain cortical neurons and although SST can modulate responses of cortical neurons to visual stimulation, results were not consistent with the expectations from modulating GABA actions, and no modulation by SST of iontophoretically applied GABA was detected. Similar relationships exist between CCK and GABA in another population of neurons in the visual cortex (40) and between NPY and NE in the hypothalamic paraventricular nucleus (PVN). The latter two substances, NPY and NE, coexist in brainstem cell groups that project to the PVN and other regions (101), and when applied into the PVN, both can induce feeding (cf 102). But in spite of these facts, the feeding-inducing action of NPY was neither a NE-dependent action, because it was not affected by the alpha-adrenergic blocker phentolamine (103, 104), nor a modulation on or synergism with NE action (105). Thus, colocalization does not necessarily lead to modulation.

Conversely, neuromodulation is not limited to colocalized substances. For instance, CCK-8 attenuated K^+-evoked DA release not only in the caudal nucleus accumbens, where CCK-8 and DA coexist, but also in the anterior portion of the nucleus, where they do not coexist (96). CCK can also modulate the activational action of DA on adenylate cyclase (99) and the number of DA binding sites (97) even where CCK and DA do not coexist.

Diversity of Neuromodulatory Actions of Single Peptides

Peptide modulation is complicated in that a neuropeptide can modulate not just one type of response from one substrate but also other types of responses from other cell types. This diversity, which is a charateristic of other peptide actions as well (106), has been observed for every widely investigated peptide and can be illustrated with the modulatory actions of CCK, SP, SST, and TRH. CCK has been shown to increase DA release from the striatum in mice (107), but to decrease DA release from caudate nucleus of cats (108) and nucleus accumbens of rats (86, 109); to increase D2 binding in nucleus accumbens (110, 111) and decrease D2 binding (decrease in Bmax but not in Kd) in the striatum (97, 112); to potentiate the actions of DA (14–17, 113) and modulate the DA agonist, apomorphine, on nigrostriatal DA neurons

(114); to potentiate the excitatory action of glutamate in the amygdala (115) and in nucleus accumbens (116); to attenuate the excitatory action of ACh in the cerebral cortex (11) and the release of ACh from caudate (72); to potentiate nicotinic ACh-evoked excitatory postsynaptic potential (EPSP) in ganglion cells (56); and to modulate opiate actions in the spinal cord (117). Substance P can attenuate the excitatory response of cortical neurons to ACh (11), the excitatory response of spinal neurons to glutamate (41, 118), and the nicotinic ACh-induced NE release from chromaffin cells (68, 69); it can potentiate the nicotinic ACh-induced depolarization in inferior mesenteric ganglion neurons (42) and the ACh-induced desensitization in PC12 (119) and chromaffin cells (66). SST can potentiate the excitatory action of ACh (39), the electrically evoked 5HT release from the cerebral cortex, hippocampus, and hypothalamus (120) and NE release from cortical slices (121); it can depress electrically or effector-induced releases of NE from the hypothalamus (92) and chromaffin cells (68, 69), of SP from cultured sensory neurons (59), and of ACh from myentric plexus (58); and it can modulate responses of visual cortical neurons to visual stimulation (40). Similarly, TRH can potentiate the excitatory responses of cortical neurons to ACh (45, 47, but see 44), ACh-induced hypertension (80), the DA-induced turning behavior (122), the effect of imipramine on forced-swimming (83), the conditioned flavor aversion (84) and the punished responding (85) induced by pentobarbital and other agents; and it can modulate neuronal responses to glutamate (43–45, 48). To a lesser degree, varieties of modulatory actions have also been observed for peptides, such as ACTH (10, 63, 123), CGRP (100, 124), and VIP (49, 50, 87, 125, 126), whose modulatory actions have not yet been as extensively studied. Of course, conditions in each assay and in the types of cells studied may account for some of the differences of modulatory actions of a given peptide. Nevertheless, it seems unlikely that these neuropeptides will be held to a single, exclusive form of modulation.

Peptides sometimes have appeared to modulate preferentially a certain type of response. Such a relationship is manifest in the many instances of the modulation of DA systems by CCK (14–17, 86, 88, 96, 97, 107–114); of ACh actions by SP (11, 18, 42, 66, 68, 69, 119) and TRH (43, 45, 47, 80, 127); and of NE actions by ACTH (10, 61, 63, 64, 79). While these preferential modulatory relations may be genuine and dictated by the mechanisms of peptide and transmitter action, at this stage they may also simply be a reflection of uneven investigation.

Is the diversity of neuromodulations an indication that a peptide can modulate through a variety of mechanisms? The answer appears to be positive. For example, CCK-8 is known to be capable of potentiating both the inhibitory action of DA (14–17, 113) and the excitatory action of glutamate (115, 116), and of attenuating the excitatory action of ACh (11). The modula-

tion of DA action can be attributed to the potentiation of binding by D_2 receptors (110, 111). But this in itself is very unlikely to be the mechanism underlying the CCK-8 modulation of the actions of glutamate and ACh. Also, by recording single neuronal activity from the ventromedial nucleus of hypothalamus in vitro, we found that although TRH could modulate the excitatory actions of all three transmitters tested (NE, ACh, and glutamate), the modulation was not uniform: In some neurons, the responses of all three transmitters were modulated, while in other neurons, TRH modulated only one or two of these transmitters and left the actions of the remaining transmitters unaffected (43). This lack of uniformity makes it seem unlikely that TRH modulated the actions of the three transmitters through a single mechanism.

Convergence of Modulatory Influences from Different Peptides

Several peptides modulating a given neural response have frequently been observed. At the neuronal level, the excitatory responses of cerebral cortical neurons to ACh have been found to be attenuated by SP, CCK-8, VIP, and AII (11) and potentiated by TRH (45, 47). Similarly, the excitatory action of glutamate can be potentiated by AVP (35, 51, 52), CCK-8 (115, 116), NT (128), OXY (35), and SST (129); attenuated by calcitonin (130), met-ENK (32, 131), ENK (132–134), and SP (41); and modulated by TRH (43–45, 48). Convergence has also been seen in the modulation of the release of growth hormone by VIP, CCK, gastrin, GRH, and galanin (see 20).

From the earlier discussion of peptide specificity in peptide modulation, it would seem that in a convergence of modulatory influences each peptide might modulate the same neural responses via different mechanisms. This could be true even when the different peptides have a similar initial effect. For instance, although SP, CGRP, and SST can all facilitate electrically-evoked hamstring flexion reflex (75, 76), the three peptides apparently employ different mechanisms. This is indicated by the findings that the modulatory actions of SP and CGRP were not merely additive but synergistic (13) and that SP but not SST can potentiate the magnitude of the flexion reflex evoked by mechanical stimulation (76). Similarly, while both SST and CCK can attenuate K^+-induced ACh release from the caudate nucleus, only SST and not CCK was mediated through DA receptors (72). Interestingly, in modulating the K^+-evoked release of amino acids, the situation was reversed; here CCK and not SST was blocked by sulpiride (90). In our laboratory, both LHRH and TRH were found to modulate the in vitro responses of preoptic neurons to NE (26). The modulatory action of LHRH can be conceived as being mediated through LHRH receptors because (*a*) the action can be duplicated by LHRH analogs (73); (*b*) many neurons in the investigated region are contacted by terminals containing LHRH-like immunoreactivity (135) and, hence, probably have LHRH receptors; (*c*) LHRH did not modulate all the neurons

responsive to NE; only some of them, probably those with LHRH receptors, were modulated (26). TRH apparently acted through something else, because none of the preoptic neurons modulated by LHRH were affected by TRH (26). In summary, in modulating a given response, different peptides may act through different mechanisns. Similar situations were also found in peptide regulations of behaviors (106).

Opposite Modulations by Individual Peptides

A peptide can modulate the response to a given neurotransmitter in opposite ways. This has been reported for CCK-8 (99, 114), ENK (62), LHRH (26), SP (42), SST (40), TRH (43), and VIP (11, 40). While at first this is puzzling, on closer examination it is obvious that the directions of dual modulatory actions are substrate dependent. In the visual cortex, for example, although both facilitation and attenuation by VIP on neuronal responses to a visual stimulus have been observed, there were differential distributions of neurons being facilitated (throughout all cortical layers) and those being attenuated (located exclusively in lamina III/IV) (40). As mentioned earlier, whether the peptide and the effector colocalize is also important. In caudal nucleus accumbens, where CCK-8 and DA colocalize, the activation of adenylate cyclase by dopamine was potentiated by CCK-8; in the anterior portion, where no colocalization was seen, modulation in the opposite direction was observed (99). Differences in a peptide's modulation have also been observed in the same type of tissue from different species. The chronotropic response of cardiac muscle induced by NE was attenuated by leu-ENK in rats but was potentiated by the same peptide in guinea pigs (62).

How does a peptide achieve opposite modulatory actions on the same type of response to the same transmitter agent? One possibility is that a transmitter can evoke the same type of responses through different mechanisms. For example, NE can excite cerebellar neurons through β-receptors (136), hypothalamic neurons through α_1-receptors (137), and hippocampal cells through α_2- and β-receptors (138). However, such cases have been rare; most neurotransmitters exert different actions when acting through different types of receptors. Besides, in the hypothalamus, where NE-evoked neuronal excitation can either be potentiated or attenuated by TRH, practically all the NE-evoked excitatory responses are mediated through α_1-receptors (137). Therefore, it is more likely that the peptide, rather than the transmitter, is responsible for dual modulatory actions.

Neuromodulation Distinct from a Peptide's Transmitter Action

It was realized early that a peptide can exert both neurotransmitter and neuromodulatory actions. For example, ACTH can excite or inhibit the activity of hippocampal cells and can attenuate the inhibitory responses of

these neurons to iontophoretically applied NE (10). Since then, many other peptides, such as AVP (35, 51, 52), CCK (14–17, 113, 114), dynorphin (DYN) (34), ENK (132–134), NPY (29), SP (41), SST (39), TRH (43, 45, 47), and VIP (50), have also been shown to be capable of acting both as a transmitter and a modulator on the same group of neurons or even on individual cells. As indicated by differences in the following characteristics, the transmitter and modulatory actions are not two modes of a single action but are independent of each other.

TIME COURSE Peptides are "slow" when acting as neurotransmitters (1, 2); they are even slower when acting as modulators. This can be illustrated by TRH actions on single-unit activity recorded from hypothalamic tissue slices (43). Identical applications of TRH can stimulate neuronal activity or modulate neuronal responses to classical neurotransmitters. While the stimulatory action may occur within one minute after application and may last 2–3 min, the modulation typically lasted for 45 min to over one hour (43). A similar contrast in duration has also been observed for iontophoretically applied AVP: Its excitatory action lasted only as long as the peptide was being applied (measured in seconds) (35), but its potentiation of glutamate-evoked excitation outlasted AVP application by up to 15 min (51). Likewise, long duration of neuromodulation has been reported for a TRH analogue, whose potentiation of electrically evoked motoneuron field potential reached a peak in 6–12 min and lasted for 30–65 min (139); for SP, whose enhancement of the amplitude of stimulus-induced excitatory postsynaptic potential reached a maximum at 5 min and lasted for up to 20 min (140); for $ACTH_{1-24}$, whose facilitatory effect on the NE-induced contraction of cardiac muscle lasted for >30 min after washout (63); and for the potentiation of a noxious flexion reflex by SP+CGRP (13) and by SST+CGRP (77) that could last for 40 min to more than an hour and half. In these cases, it is obvious that the duration of the modulatory actions is longer than that expected for transmitter actions. To our knowledge, the only exception to the slow time course is the rapid (in both onset and recovery) modulation by SP on responses of cultured spinal neurons to glutamate excitation (41).

LACK OF CAUSAL RELATIONSHIP There are cases where the transmitter and modulatory actions of a peptide are opposite in direction. For instance, in the nucleus accumbens, CCK can stimulate neuronal activity on one hand and potentiate the inhibitory action of DA and apomorphine on the other (14–16). In these cases the lack of a causal relationship between the two peptide actions is obvious. Even in the cases where both actions of a peptide are in the same direction, the transmitter and modulatory actions are also evidently independent of each other. For example, in the septal region, AVP can either

excite neurons directly or potentiate the excitatory action of glutamate, but it can exert the modulatory action on neurons that cannot be stimulated by the peptide itself (35, 51), which indicates that the modulatory action does not require the occurrence of a transmitter-like action. This indication is further supported by the findings that, on ganglion cells, CCK (56) and SP (42) can cause the potentiation of an nACh-evoked EPSP, even when their direct depolarizing action has been neutralized. In our study on TRH (43), we found that a neuron can be modulated by TRH, regardless of whether it is also stimulated by the peptide (n = 8 units) or not (n = 19). The converse is also true, that a neuron can be stimulated by TRH, regardless of whether it is modulated by the peptide. These observations indicate that neither action is a necessary consequence of the other.

DESENSITIZATION On repeated applications the transmitter action of many peptides desensitizes rapidly (for desensitization of other peptide actions see Ref. 106). This has been observed for AII (141), AVP (142), CCK (143, 144), OXY (142), SP (41), SST (145), TRH (43), and VIP (146, 147). In contrast, to our knowledge, only one definite case of densensitization has been reported for modulation (58). This contrast regarding desensitization has been clearly shown by the action of SP on cultured spinal neurons (41). On a given neuron, this peptide can cause both neuronal excitation and the attenuation of the excitatory response to glutamate; and while its excitatory action disappeared upon repeated application, it still continued to modulate glutamate action. Obviously, the transmitter and the modulatory actions were not mediated through the same mechanisms.

SENSITIVITY TO PEPTIDE In applying AVP iontophoretically, generally 100–150 nA were required to evoke neuronal excitation (51), but to achieve modulation required only 20–50 nA (52). By varying the distance between the tip of the ENK-releasing micropipette and the responding neuron, it was found that modulation of a glutamate response could be achieved at a longer distance (and hence greater dilution) than that required for the activation of membrane conductance (134). Similar situations have been reported for NPY (29, 30), SST (39), and VIP (50). Such observations indicate that the dose of a peptide required for evoking modulatory action was lower than that for transmitter action. Interestingly, in no case did the modulatory action require a higher dose.

ACTIVITY OF METABOLITES In the study of TRH and its metabolite, cHP, it was found that cHP shared the modulatory, but not the stimulatory, action of TRH on hypothalamic neurons (43). This, together with the findings that cHP does not bind to TRH receptors (148, 149), suggests that TRH acts through

classical TRH receptors to stimulate neuronal activity but through other, still undefined mechanisms to modulate neuronal responses. A similar suggestion has been proposed on the basis of comparisons between the actions of sulfated and nonsulfated forms of CCK-8 (17, 113).

The independence of the modulatory and the transmitter-like actions indicated by these characteristic differences makes it possible for these two kinds of actions to mediate different biological effects of a peptide.

MECHANISMS FOR PEPTIDE MODULATION

Mechanisms for neuromodulation in general (8) and for the modulation of transmitter release in particular (150) have been reviewed elsewhere. Here, we focus on the mechanisms underlying modulations by peptides. From the characteristics of peptide modulation discussed above, several suggestions can be derived. Lines of evidence for specificity make it hard to conceive that all peptides achieve their neuromodulations through a single, general mechanism.

Alterations of Transmitter Binding

An obvious possibility for modulating the responses of neurons to a neurotransmitter is the induction of a change in the binding of the transmitter. Therefore, the effects of peptides on binding have been intensively investigated, and many peptides have been found to cause changes in the number of binding sites (Bmax) and/or the affinity (Kd). These peptides include ACTH on muscarinic binding (151), CCK on DA binding (97, 111, 112), CGRP on ACh binding (124), NPY on α_2-adrenergic binding (152), NT on DA binding (153), opiates on TRH binding (154), PRL on DA binding (36), SP on ACh (119, 155) and $5HT_1$ binding (156), TRH on ACh (65, 80) and $5HT_1$ binding (157), and VIP on $5HT_1$ binding (125, 126). In almost every case, the number of binding sites (Bmax) was altered, mostly by an increase. In a few cases the affinity, either alone or together with Bmax, was also changed, usually by a decrease.

A neurotransmitter usually can bind to different types of receptors that mediate different actions, and such different receptor types can exist on individual hypothalamic neurons (137). Therefore, even with alteration of only one type of receptor, a peptide may cause a complex modulation by changing the ratio of different types of receptors and, hence, the balance of different kinds of actions of that transmitter.

The alteration of binding is, of course, not the ultimate mechanism for peptide neuromodulation, but can, in turn, be the result of an underlying cellular change. In the case of Bmax increases, it is unlikely that peptides achieve this by inducing the synthesis of receptors, because most of the

increases were induced by peptides in relatively short periods of time (minutes). This is supported by the findings that neither AVP, LHRH, SST, nor TRH induced RNA synthesis in calf pituitary, brain cortex, or liver, or in rat brain nuclei (158). Other mechanisms may include competitive binding in the reduction of affinity (151), allosteric interactions (119, 155), receptor-receptor interactions (cf 159), and changes in membrane fluidity (see below). These mechanisms are not well understood, and more investigations are required.

Second Messengers

Many peptides have been shown to be capable of modifying second messenger systems, e.g. adenylate cyclase-cAMP and membrane phospholipid systems. A notable example is the alteration of cAMP levels in certain hypothalamic nuclei by VIP and several other peptides (160, 161). Other examples can be found in recent reviews (cf 2, 162, 163). Coupled with this, there are reports that second messengers can alter neural responses or behaviors, such as the augmentation of evoked transmitter release by protein kinase C (164), potentiation of synaptic transmission by a protein kinase C activator (a phorbol ester) (165), and the induction of behaviors by a cAMP analog (166). Therefore, it seems probable that peptides can act through second messengers to modulate neural or behavioral responses to a stimulation or transmitter. In pancreatic acinar cells, CCK appears to act through the second messenger, Ca^{2+}, to activate a nonselective cation channel to induce secretion (167), but whether this mechanism can also account for the modulatory action of CCK is still unknown. In some cases, evidence has shown that the modulatory action of a peptide was *independent* of the second messenger system involved in mediating the peptide's direct action. For instance, although VIP can stimulate the synthesis of cAMP (160, 161), its attenuation of CCK release has been shown to be independent of the activation of the adenylate cyclase (87). Similarly, ACTH is capable of affecting the adenylate-cyclase-cAMP system (168), but its potentiation of NE action on atrial tissue did not involve changes in cAMP (64). Nevertheless, there are hints that peptides can act through secondary messengers to modulate neural responses. These include suggestions that SST attenuates the DA-induced activation of adenylate cyclase by binding to an inhibitory subunit of regulatory adeylate cyclase components (71); that TRH modulates the DA-induced turning behavior and the activation of cAMP synthesis by affecting the adenylate cyclase (122); that ACTH modulates behaviors through membrane phospholipids (169); and that AVP potentiated NE-induced activation of cAMP synthesis through Ca^{2+}/calmodulin (170). Thus, although the evidence is still thin, the modification of second messengers remains a potential mechanism for peptide neuromodulation.

Membrane Potential

As discussed in the section on response specificity, a peptide can modulate the response of a given neuron to a neurotransmitter without equally modulating the response of the same neuron to another transmitter. For example, AVP can potentiate the excitatory response of lateral septal neurons to glutamate, but it leaves the inhibitory responses of the same neurons unaffected (51); and, on a single hypothalamic neuron, TRH can potentiate the excitatory response to glutamate, but cannot potentiate a similar response to ACh (43). From this specificity, it can be inferred, even without intracellular recording, that peptides do not necessarily modulate neuronal responses by simply raising or lowering the membrane potential of the target neuron.

Indeed, the above inference has been shown directly with intracellular recording experiments. On cultured spinal neurons, SP can both increase neuronal activity and attenuate the excitatory neuronal response to glutamate (41). The former, transmitter-like action was accompanied by a depolarization and an increase in membrane conductance that desensitized quickly. When desensitization was induced to prevent any detectable change in membrane potential or conductance, SP could *still* modulate the neuronal response to glutamate (41). Consistent with this is the observation that calcitonin (CT)/CGRP can prolong the duration of after-hyperpolarization but has no effect on resting membrane potential or input resistance (171). Similarly, the small depolarization accompanying the SP- or CCK-induced potentiation of a nicotinic response can be nullified without abolishing modulation (42, 56). Another type of neuromodulation, attenuation of K^+-induced release of SP from cultured dorsal root ganglion cells by SST or ENK, has also been reported to take place without a change in the resting membrane potential (59). In a few cases, however, changes in membrane potential may be involved in peptide neuromodulation (57, 74).

Regulation of Ion Channels

Many peptides are capable of modifying ion channels, whose changes obviously can alter cellular functions such as electrical excitability, firing patterns, duration of excitation, etc (8, 172). A notable example is the inhibition of the M-current, a K^+-current known to be inhibited by muscarinic agonists, by LHRH (173, 174), and SP (57, 175). Since the inhibition of the M-current can change neuronal excitability and firing patterns (cf 172), LHRH and SP can thereby modulate neuronal responses. In an opposite manner, opioid peptides, probably acting through mu and delta receptors (176, 177), can activate a voltage-sensitive K^+ channel, and thereby decrease Ca^{2+} influx or the duration of a Ca^{2+} spike (176, 178). Such an effect can inhibit Ca^{2+}-dependent release. Indeed, this mechanism has been proposed to account for the attenuation of K^+-evoked SP release by ENK and SST (59)

and the attenuation of the NE-induced chronotropic response in rat atria by len-ENK (62). The effects of CT/CGRP to prolong the duration of the action potential and after-hyperpolarization potential without affecting resting membrane potential, input resistance, or the amplitude of the action potential suggest that the peptides can also regulate ion channels, probably Ca^{2+} or Ca^{2+}-dependent K^+ channels (171). Peptides can also regulate ion channels in endocrine cells, such as TRH on K^+ (179) and voltage-dependent Ca^{2+} channels (180) in pituitary cells; SP on channels opened by ACh in chromaffin cells (66); and CCK on nonselect cation channels in pancreatic acinar cells (167). These regulations may be involved in the modulation of endocrine secretions. It appears that in neurons, modulation of K^+ currents will continue to be a fertile subject for studying mechanisms of peptide actions. Consequently, guanine nucleotide-binding proteins, which can regulate K^+-channels without affecting the resting membrane potential or conductance (181), are potentially important.

Questions About Receptor-Mediated Mechanisms

Because of the scarcity of specific antagonists, the involvement of classical receptors in peptide neuromodulation has been difficult to prove directly. There are indirect indications: We found that the modulatory action of LHRH can be duplicated by LHRH analogs but not by a control peptide of similar length and secondary structure nor by another neuromodulator, TRH (26, 73). Such evidence as discussed under the section on specificity (above) strongly suggests the involvement of specific receptors in peptide neuromodulation. However, there are also contrary indications. For example, VIP achieves its biological actions by acting on specific VIP receptors, apparently coupled to the adenylate cyclase-cAMP system, to stimulate cAMP synthesis (cf 87). Yet, in the attenuation of K^+-evoked CCK release, the modulatory action of VIP appears to be independent of the adenylate cyclase-cAMP system (87). Therefore, it is possible that in this modulatory effect VIP does not act through VIP receptors. The lack of specific receptor involvement is also a possible explanation for the failures of proglumide, a CCK receptor antagonist, to block the modulatory action of CCK-8 in the attenuation of DA release (109) and of a kappa-opioid receptor antagonist to block a modulatory action of DYN (34). Furthermore, although no specific binding in the brain has been found for ACTH (151) or the TRH metabolite cHP (148, 149), both these peptides are capable of modulating neuronal responses [ACTH (10); cHP (43, 45)]. Of course, these peptides may bind to as yet undiscovered receptors, but until these are established we should consider ways in which peptides can modulate neural responses without acting through specific receptors.

One such way may be the alteration of membrane fluidity, as indicated by

the following two lines of evidence. First, many peptides such as TRH, NT, BBS, and β-END are capable of modifying various ethanol-induced behavioral changes (182–185). The exact mechanism(s) for the peptide-ethanol interactions are not known. But, since ethanol does not act through specific receptors and can act through selective perturbation of membrane fluidity (186), it is possible that, in modifying ethanol-induced behavioral changes, peptides also affect membrane fluidity. Secondly, changes in membrane fluidity can alter the receptor-effector coupling and/or binding parameters of adrenergic, muscarinic, nicotinic, serotonergic, and GABAergic receptors and receptors for many peptides (187). Thus, by modifying membrane fluidity, peptides can exert a wide variety of modulatory actions on neural responses.

Another way a peptide can exert its neuromodulatory action is derived from chemical studies of peptide binding to transmitters. In one study, it was found that LHRH and MSH-ACTH possessed a 5HT binding site sequence and could bind 5HT, but not other bioactive amines (188). Another study found that LHRH could bind, in a sequence and residue-specific way, to a tripeptide (189), which attenuates LHRH-induced release of FSH (190). These studies suggest that a peptide can modulate the action of neurotransmitters and other peptides by directly binding to them. Since such binding could include forces between amino acids (189), a peptide could bind to proteins such as transmitter-related enzymes. Such may be the mechanism, for example, underlying the interesting observation that CGRP can potentiate and prolong the action of SP by interacting with the enzyme that degrades SP (191).

Involvement of Glial Cells

There is evidence that substances secreted from a neuron can affect the activity of glial cells, which, in turn, can modify the activity of the same or other neurons (192). The secreted substances can be peptides, because peptide-containing nerve terminals have been observed apposed to astrocytes (193, 194). These observations, together with reports that a variety of peptides can directly stimulate or indirectly modulate the level of glial cAMP (70, 195), make it conceivable that glial cells can participate in some of the neuromodulatory actions of peptides.

NEUROMODULATION AS A MECHANISM FOR PEPTIDE EFFECTS

On Behaviors

By acting on subcellular elements in the nervous system, certain peptides may eventually affect behaviors. A full review of peptide-behavior relations is outside the scope of this chapter, but some principles summarizing parts of

that literature have been published recently (106). It is often the modulatory action, rather than the transmitter action, that underlies the behavioral effect of a peptide. For instance, insulin could act centrally to exert a dose-related inhibition of food intake and body weight (cf 196). Yet, this peptide did not affect the spontaneous activity of the glucose-responsive neurons in the ventromedial nucleus of the hypothalamus that are relevant for controlling feeding (23). Instead, the peptide potentiated the responses of these feeding-relevant neurons to glucose (23), which suggests that insulin regulates food intake and body weight by neuromodulation. This suggestion is further supported by the finding that intracisternal infusion of insulin, at a dose having no effect on food intake, enhanced the suppressive effect of CCK-8 on meal size (22). Similar conclusions can be derived from our findings that both TRH and its metabolite cHP, which share an anorexic effect (197, 198), can modulate transmitter-evoked hypothalamic neuronal responses, but only TRH can stimulate the spontaneous activity of VMN neurons (43). Consistent with these findings is the report that FMRFamide can suppress feeding behavior induced by exogenous or endogenous opioids (19).

Peptide modulation may also play a role in the regulation of other behaviors, such as the modulation of lordosis behavior by LHRH (199), PRL (200), SP (201), β-END (202), and CRF (203); the facilitation of learning and memory by AVP through its inhibitory modulation on NE-induced cAMP accumulation (89); the potentiation of DA-mediated behaviors by CCK through CCK modulation of the mesolimbic DA system (88); the suppression of d-amphetamine-induced hyperlocomotion by NT through its modulation of the mesolimbic DA system (31); the facilitation by SP and attenuation by CRF of a carbachol-induced seizure behavior (18); and the action on the sleep-waking cycle by VIP through its modulation of binding of $5HT_1$ receptors (cf 204).

On Autonomic and Neuroendocrine Functions

Neuromodulation also appears to mediate certain peptide effects on functions such as blood pressure, hormone release, body temperature, etc. The involvement of peptide modulation in the regulation of blood pressure was indicated by observations that, at subpressor doses, peptides such as ACTH (79), AII (205), NPY (29), and TRH (80) could potentiate the pressor effects of NE and ACh. A report that AVP attenuated [^{3}H]NE release only in normotensive and not in spontaneously hypertensive rats (206) implies that AVP, too, can help to regulate blood pressure through a modulatory action. Neuropeptide modulations may also be involved in the regulation of body temperature by AVP (81, 82); LHRH release by met-ENK (33); the release of luteinizing hormone by NPY (28); and gastrointestinal functions by NPY (78) and TRH (91). The demonstration of synaptic relationships between LHRH-containing nerve ter-

minals and perikarya in the preoptic area of the hypothalamus (POA) (135), together with findings that LHRH acts more as a neuromodulator than a transmitter on POA neurons in vitro (26), raises the possibility that LHRH may exert an endocrine feedback effect through neuromodulation.

In summary, it is clear that neuromodulation is widely involved in the peptide regulation of neural and endocrine functions. Thus, not limiting investigations to transmitter-like actions and searching for modulatory effects will help not only in the dissection of neuropeptide mechanisms but also in the development of drugs that interact with peptide-responsive neural systems.

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