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Phosphoramidon Potentiates Mammalian Tachykinin-Induced Biting, Licking and Scratching Behaviour in Mice

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SAKURADA, T., K. TAN-NO, T. YAMADA, S. SAKURADA AND K. KISARA. *Phosphoramidon potentiates mammalian tachykinin-induced biting, licking and scratching behaviour in mice.* PHARMACOL BIOCHEM BEHAV **37**(4) 779–783, 1990.— The effects of peptidase inhibitors were examined upon behavioural responses including scratch, bite and lick produced by intrathecal (IT) injection of substance P (SP) and neurokinin A (NK A) in mice. Phosphoramidon (0.002–2.0 nmol), an endopeptidase-24.11 inhibitor, simultaneously injected with SP or NK A, remarkably enhanced and prolonged SP- or NK A-induced behavioural response in a dose-dependent manner. The behavioural response to SP was significantly increased by 2.0 nmol of bestatin, an aminopeptidase inhibitor, but not by 1.0 nmol. Captopril, an angiotensin-converting enzyme inhibitor, was without effect on both tachykinin-induced responses. When phosphoramidon was injected together with bestatin and captopril which have no significant effect alone, SP- or NK A-induced behavioural response was significantly increased. These data suggest that endopeptidase-24.11 may be an important enzyme responsible for terminating of SP- or NK A-induced behavioural response at the spinal cord level.

Substance P Neurokinin A Intrathecal injection Phosphoramidon Bestatin Captopril Aversive response

SUBSTANCE P (SP) localized in the primary afferent nerve fibers has been suggested to function as a neurotransmitter in the dorsal horn of the spinal cord. SP was initially identified in 1931 (5) and two other tachykinins have fairly recently been described in mammalian central nervous system. These have been termed NK A and NK B which were found in different laboratories as neurokinin (10), neuromedin K (9) and neuromedin L (16).

It has been demonstrated that intrathecally (IT) administered SP elicits a characteristic behaviour in conscious mice and rats suggestive of the perception of peripheral irritation. The behavioural response induced by SP was reduced by several endogenous opioid peptides and inversely potentiated by naloxone, an opioid antagonist (27). Thus, it appears that endogenous opioid peptides, particularly Met-enkephalins, interact with SP-induced response in the spinal cord level (23). IT injected NK A can induce similar behavioural response to SP, though it was considerably less potent than SP (6, 25, 30).

Several studies on the enzymatic degradation of neuropeptides indicate that membrane metalloendopeptidase (enkephalinase) (14,26) and peptidyl dipeptidase A (angiotensin-converting enzyme) (32) are capable of cleaving SP. NK A is also hydrolysed by the synaptic membrane endopeptidase which can degrade SP (7). If these enzymes act on the degradation of SP and NK A in the spinal cord, we would expect the possibility that an endopeptidase-24.11 inhibitor phosphoramidon, an angiotensin-converting enzyme inhibitor captopril , or an aminopeptidase inhibitor bestatin can potentiate the behavioural response elicited by IT injection of SP or NK A.

In the present study, we have investigated the effect of peptidase inhibitors involved in the degradation of endogenous tachykinins on SP- or NK A-induced behavioural response in mice.

METHOD

Subjects

Male Std-ddY mice, weighing 20–24 g, were housed in colony cages at room temperature of 23 ± 2°C and humidity 55 ± 5% in a room with a 12-hr light dark cycle. They had free access to water and food. The experimental groups were chosen at random. All experiments were performed between 10:00 and 15:00 to avoid possible circadian variation.

Intrathecal Injections

IT injections were carried out by lumbar puncture through intact skin in unanesthetized mice, essentially as described by Hylden and Wilcox (8). Briefly, a lumbar puncture was performed using a 29-gauge needle connected to a Hamilton microsyringe. The needle was directly inserted between the L5 and L6 vertebrae and a rapid IT injection of 5 μl was then made. The accurate placement of the injections was indicated by a quick flick of the mouse's tail. SP, NK A, phosphoramidon and bestatin were purchased from Peptide Institute Inc. (Osaka). Captopril was

a gift from Sakyo Co. Inc. (Tokyo). These drugs injected IT were dissolved in sterile artificial cerebrospinal fluid (CSF).

Behavioural Assessment

The animals were adapted to standard transparent cages (22.0 × 15.0 × 12.5 cm) one hour prior to IT injection. Immediately following IT injections, the mice were placed in the individual cage and the total time spent for reciprocal hindlimb scratching and hindpaw licking/biting episodes was measured every 5 min for 20 min.

Statistics

The results were treated by a one-way analysis of variance (ANOVA). This was followed by Dunnett's test. A difference was considered statistically significant at $p<0.05$. The values are expressed as the mean ± the standard error (SEM).

RESULTS

Effects of Phosphoramidon on SP- and NK A-Induced Behavioural Response

SP (0.1 nmol) or NK A (0.4 nmol) resulted in caudally biting, licking and scratching behaviour in mice following their IT injections. This characteristic behaviour was evident at 5 min following injection and had subsided gradually afterwards (Fig. 1). When simultaneously injected with phosphoramidon (0.02–2.0 nmol), SP- or NK A-induced behavioural response was markedly enhanced even at 15–20 min postinjection. During the 20-min observation, the total response time of SP or NK A was elevated by coadministration of phosphoramidon (0.002–2.0 nmol), as shown by the dose-dependent increase (Fig. 2). CSF had no effect on behaviour when injected alone.

Effects of Bestatin, Captopril and in Combination With Phosphoramidon on SP- and NK A-Induced Behavioural Response

SP-induced behavioural response was not significantly enhanced by 1.0 nmol of bestatin (Fig. 3, upper panel). A relatively high dose (2.0 nmol) of bestatin was significantly effective in producing SP-induced response (92.0 ± 12.0 sec/20 min, $p<0.01$ as compared to SP alone). The same dose of bestatin was without significant effect on NK A-induced response (Fig. 3, lower panel). Inhibition of an angiotensin-converting enzyme by captopril (2.0 nmol) did not significantly alter SP- or NK A-induced response. IT injected phosphoramidon, bestatin or captopril alone produced no notable behavioural response which was approximately the same value with artificial CSF control (4.0 ± 1.9 sec/20 min). Coadministration of bestatin (1.0 nmol for SP and 2.0 nmol for NK A), captopril (2.0 nmol) and phosphoramidon (0.002 nmol), which had no significant effect on each agonist, produced a statistically significant increase of SP or NK A (Fig. 3).

As shown in Fig. 4 (upper panel), enhanced effects of SP by phosphoramidon (0.02 nmol) were significantly potentiated by coadministration of bestatin (1.0 nmol) or in combination with captopril (2.0 nmol). Captopril (2.0 nmol) was without effect on SP-induced enhancement by phosphoramidon. Phosphoramidon (0.02 nmol) produced a significant increase of NK A-induced response and this effect was potentiated by simultaneous administration of two peptidase inhibitors, bestatin (2.0 nmol) and captopril (2.0 nmol) (Fig. 4, lower panel). The effect of NK A by phosphoramidon was not significantly changed by either bestatin or captopril.

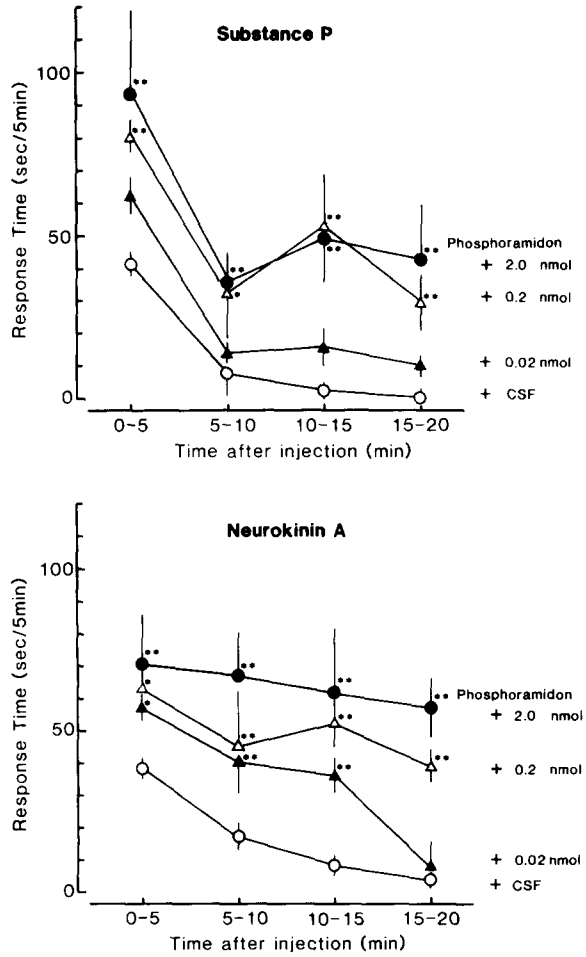
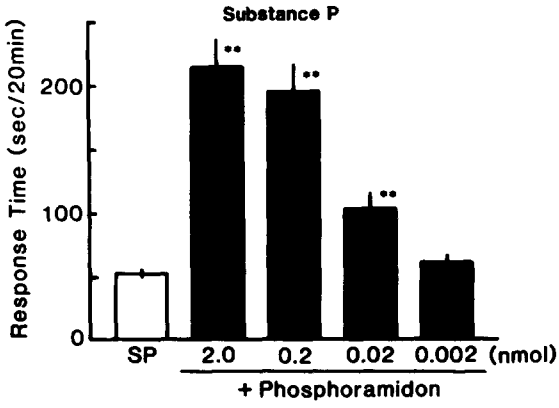


FIG. 1. Time course effect of phosphoramidon on substance P- and neurokinin A-induced aversive behaviour in mice. Mice were coadministered CSF or various doses of phosphoramidon in combination with 0.1 nmol of substance P (SP) or 0.4 nmol of neurokinin A (NK A). The data are given as means ± S.E. for groups of 10 mice. $^{**}p<0.01$, $^{*}p<0.05$, when compared to SP or NK A alone.

DISCUSSION

The present results confirm those of earlier study showing that IT injections of both mammalian tachykinins, SP and NK A, resulted in a behavioural syndrome characterized by a licking, biting and scratching episode in mice (6, 8, 25, 27). Like SP, IT injection of NK A also caused this characteristic behavioural response, though its potency and mechanism of action have been considered to be different from SP. SP and the other mammalian tachykinins, NK A and NK B, have diverse pharmacological actions in various systems (21). The existence of multiple tachykinin receptors was proposed in mammalian tissues based upon their rank order of potency (12). These receptors have been designated NK-1, which preferentially binds SP, and NK-2, which is selective for NK A. A NK-3 receptor has also been described and preferentially binds NK B. On the basis of the rank order of potency of various tachykinins for inducing the biting, licking and



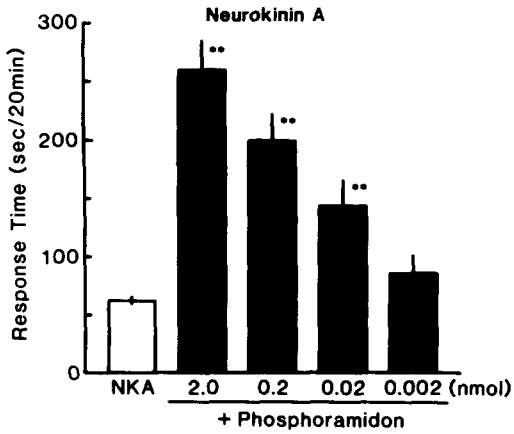
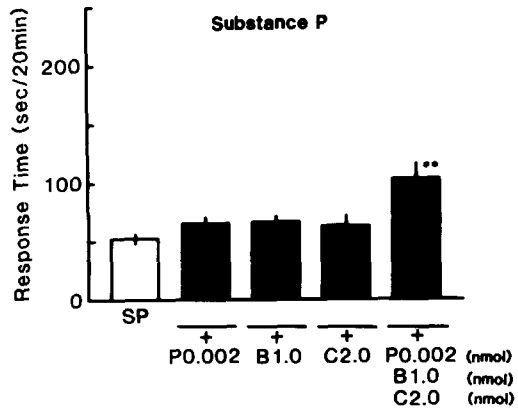


FIG. 2. Dose-related increase of phosphoramidon on substance P- and neurokinin A-induced aversive response in mice. Mice were coadministered CSF or various doses of phosphoramidon in combination with 0.1 nmol of substance P (SP) or 0.4 nmol of neurokinin A (NK A). The data are given as means ± S.E. for groups of 10 mice. $^{**}p<0.01$, when compared to SP or NK A alone.



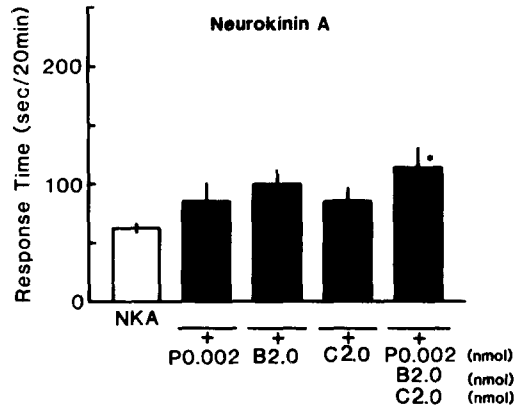


FIG. 3. Effects of three peptidase inhibitors on substance P- and neurokinin A-induced aversive behaviour in mice. Mice were coadministered CSF, phosphoramidon (P), bestatin (B), captopril (C) or three peptidase inhibitors in combination with 0.1 nmol of substance P (SP) or 0.4 nmol of neurokinin A (NK A). The data are given as means ± S.E. for groups of 10 mice. $^{**}p<0.01$, $^{*}p<0.05$, when compared to SP or NK A alone.

scratching behaviour, the spinally mediated behavioural response was found to be chiefly mediated through NK-1 receptors in the spinal cord (25).

SP- or NK A-induced behavioural response was rapid in onset and short-lasting following IT injection. It seems evident that the short duration of SP and NK A is due to rapid metabolism of the peptides by enzymes existing in the central nervous system (7,14). SP is cleaved by prolylendopeptidase to form an N-terminal tetrapeptide and a C-terminal heptapeptide (1). Some of both C- and N-terminal fragments possess biological activity. The SP heptapeptide is as potent as SP or even more potent than SP in contracting the guinea pig ileum (31), in depolarizing rat motoneurones (20) and in inducing biting, licking and scratching behaviour (24). Moreover, several membrane-bound enzymes capable of hydrolyzing SP and NK A have been identified from central nervous tissues (7,14). Several endopeptidases from human brain hydrolyse SP at peptide bonds between Gln^6-Phe^7, Phe^7-Phe^8 and Phe^8-Gly^9 or Gly^9-Leu^{11} (11) and NK A at positions between Gly^8-Leu^9 and Leu^9-Met^{10} (7). Regarding the endopeptidase in human CSF, Nyberg et al. (18) reported that a SP-hydrolyzing endopeptidase purified by human cerebrospinal fluid can cleave predominantly at the Phe^7-Phe^8 and Phe^8-Gly^9 bonds. In the substantia nigra, phosphoramidon, which is known as a specific inhibitor of endopeptidase-24.11, selectively inhibited SP degradation by synaptic membrane (19). In the present study, SP- or NK A-induced response was markedly potentiated and prolonged by coadministration of phosphoramidon, an inhibitor of endopeptidase-24.11. The data support the involvement of endopeptidase-24.11 as an enzyme that is important in the spinal catabolism of SP and NK A. On the other hand, the behavioural response to both mammalian tachykinins was not significantly changed by 1.0 nmol of bestatin and 2.0 nmol of captopril. Therefore, aminopeptidases and angiotensin-converting enzymes do not play predominant roles in modulating the spinal action of SP and NK A. Taking account of previously reported results, the present behavioural data indicate that a phosphoramidon-sensitive enzyme may be a major endopeptidase implicated in the degradation of SP and NK A. This enzyme can hydrolyze a variety of neuropeptides such as a nonmammalian tachykinin, physalaemin as well as SP and NK A (15), neurotensin, cholecystokinin (29) and enkephalins (13). In the peripheral tissues, there is evidence that neutral endopeptidases purified from pig and human kidney (14,26) and pig small intestine (17) cleaved at amino side of hydrophobic amino acids and formed SP (1–6), SP (1–7) and SP

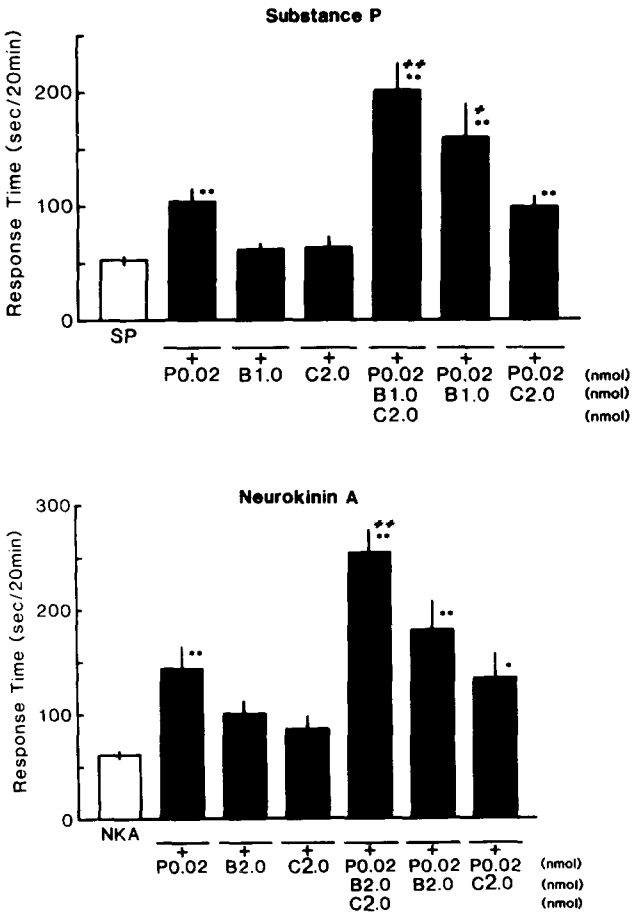


FIG. 4. Effects of bestatin, captopril and in combination with phosphoramidon on substance P- and neurokinin A-induced aversive behaviour in mice. Mice were coadministered CSF, each peptidase inhibitor, phosphoramidon (P) plus bestatin (B), phosphoramidon (P) plus captopril (C) or three peptidase inhibitors. The data are given as means ± S.E. for groups of 10 mice. $**p<0.01$, $*p<0.05$, when compared to substance P (SP) or neurokinin A (NK A) alone. $\#\#p<0.01$, $\#p<0.05$, when compared to SP or NK A plus phosphoramidon (P).

(1–8) as major metabolites. Phosphoramidon completely abolished the cleavage of SP by these endopeptidases. Therefore, it is presumed that SP N-terminal fragments, SP (1–6), (1–7) and (1–8) arise from the degradation of SP by endopeptidase in the spinal cord. Immunoreactive SP (1–7) was also established to be highest in the dorsal part of spinal cord as well as SP (1–11) (22). Moreover, it should be noted that the degraded product of SP, (1–7) antagonizes SP action in the spinal cord (24), whereas SP (1–7) was without effect on the activity of the guinea pig ileum (3).

A combination of phosphoramidon and bestatin produced a significant increase in the SP-induced response, indicating that a bestatin-sensitive aminopeptidase may partially contribute to the hydrolysis of SP. On the other hand, SP and NK A are known to be endogenous substrate for angiotensin-converting enzyme in the brain (28,32). Inhibitors of peptidyl dipeptidase A such as captopril have been shown to potentiate certain peripheral actions of SP, such as salivation (2), suggesting that this enzyme might also play a role in SP metabolism in vivo, at least in peripheral tissues. However, it appears that SP and NK A are not degraded by an angiotensin-converting enzyme in the spinal cord, since captopril produced no significant effect on behavioural responses produced by SP and NK A. This enzyme may not be also involved in the degradation of NK A injected IT, judging from the present behavioural data. Finally, the possibility should be mentioned that the potency of the enzyme inhibitors may differ merely because these drugs are absorbed, distributed or metabolized at different rates and not because they produce a selective effect on a particular enzyme.

In conclusion, we have demonstrated that SP- or NK A-induced behavioural response was potently potentiated by phosphoramidon. The result suggests that endopeptidase-24.11 may play a significant role in terminating the behavioural response produced by IT injection of SP and NK A in mice.

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