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The modulatory role of neurokinins in affective behaviors

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Neurokinins are peptide molecules with modulatory actions on other neurotransmitter systems, notably the monoaminergic ones, within the CNS and peripheral tissues. A great deal of evidence supports a role for these substances, mainly for substance P and its main receptor NK_1, in a number of physiologic and pathologic conditions, including affective and behavioral responses to stress. NK_1 receptor antagonists have shown preclinical activity in several paradigms of anxiety and depression. Mutant mice lacking the NK_1 receptor gene have an increased firing rate of dorsal raphe serotonergic neurons, an effect that can also be seen after the administration of substance P antagonists. When given chronically, NK_1 antagonists promote an enhancement of serotonergic transmission in the hippocampus that seems to be mediated by interaction with other neurotransmission systems. Clinical efficacy of such drugs has also been demonstrated among patients with major depression, although the results have been inconclusive. More research is needed to elucidate the precise role these drugs could play in the treatment of affective disorders in the future.

Key words:
Neurokinin. Substance P. Depression. Anxiety. Review.

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El papel modulador de las neuroquininas en las conductas afectivas

Las neuroquininas son moléculas peptídicas que tienen una acción moduladora de otros sistemas neurotransmisores, entre ellos los monoaminérgicos, en el sistema nervioso central, así como en tejidos periféricos. Existen numerosas evidencias de que estas sustancias, y en especial la sustancia P y su principal receptor NK_1, intervienen en numerosos procesos fisiológicos y patológicos, entre ellos las respuestas conductuales y afectivas al estrés. Los antagonistas del receptor NK_1 han mostrado actividad preclínica en diversos modelos de ansiedad y de depresión. Los ratones mutantes con ausencia del gen del receptor NK_1 presentan un aumento de la frecuencia de descarga de las neuronas serotoninérgicas del rafe dorsal, un efecto que también se observa tras la administración de antagonistas de la sustancia P. La administración crónica de antagonistas NK_1 produce un aumento de la transmisión serotoninérgica en el hipocampo, que está mediada por la interacción con otros sistemas de neurotransmisión. También se ha demostrado la eficacia clínica de estos fármacos en pacientes con depresión mayor, aunque los resultados no han sido concluyentes. Son precisos más estudios para aclarar el papel que puede desempeñar este tipo de fármacos en el tratamiento de los trastornos afectivos en el futuro.

Palabras clave:
Neuroquininas. Sustancia P. Depresión. Ansiedad. Revisión.

INTRODUCTION

Since more than 40 years ago, the activity of most of the drugs used in the treatment of anxiety and depression is based on the modulation of the different mechanisms that regulate the activity of gabaergic and other monoaminergic systems. However, in recent years, possible new targets have been identified for these drugs, among them tachykinins, a group of peptide substances that share the property of producing a rapid contraction of the smooth muscle and that also receive the name of neurokinins (NK) in the mammalian central nervous system (CNS). After the discovery of the first one of them, substance P (SP), in 1931, it was soon seen that it was preferably located in the spinal cord, especially in the posterior roots, and it was rapidly suspected that it played an important role in pain perception[1]. Since then, there has been an important advance in the elucidation of the role played by SP in the transmission of painful stimuli[1,2] and an attempt has been made to develop analgesics based on the blockade of its receptors. However, even though the progress on the knowledge regarding the invol-

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vement of SP in the nociceptive mechanisms has been considerable during these years, all the research efforts to obtain new drugs oriented towards this target have been unsuccessful. This may be due to the fact that the actions of the neuropeptides are only modulatory and thus have less significance and are more difficult to demonstrate than those of the classical neurotransmitters. It has also been suggested that neuropeptides could be redundant molecules, which have lost their importance in higher organisms[3,4]. Whatever, the reality is that SP antagonists have failed as analgesics[4,5], probably because these drugs are capable of lessening the body response to stressing stimuli, but this attenuation is not enough to produce analgesia since a more complete blockade of the sensorial afferences to the CNS is necessary for it[4]. In recent years, progress in the knowledge of the function played by SP and other NK in physiological and pathological conditions has been extended to other fields, NK being involved in conditions such as vomiting, cough, or inflammatory conditions, such as asthma or irritable bowel syndrome and in the physiopathology of different neurological and psychiatric diseases, such as schizophrenia, Parkinson's disease, multiple sclerosis or Alzheimer's disease[6]. One of these fields to which the study of a possible physiopathological role of NK has been extended to is that of depressive disorders and anxiety, since there is a great deal of evidence that NK, and especially SP, participate in behavioral and affective responses to stress. The present article summarizes the current status of the research regarding NK as mediators in anxiety and depression mechanisms and to the development of antagonist drugs of these neuropeptides.

NEUROBIOLOGY OF THE NEUROKININS

NK are a group of molecules made up of short amino acid chains that may act as neurotransmitter in the nervous system and play a still poorly known role, which is probably modulatory of the monoaminergic transmission. The first one, SP, was discovered by Von Euler and Gaddum, and was isolated from the horse intestine as white powder (which accounts for its name). Fifty years later, other similar neuropeptides were discovered. These were neurokinin A (NK-A), initially called substance K, and neurokinin B (NK-B), which, in the beginning, was designated neuromedine K. These share with SP the same carboxy-terminal amino acid sequence: Phe-X-Gly-Leu-Met-NH, being X Phe or Val[7,8]. All NK are produced through precursor peptides, synthesized in the ribosomes of the peptidergic neurons from two genes. In the mammals, SP and NK-A derive from the pre-protachykinin A (PPT-A) gene. The alternative transcription of the PPT-A gene gives rise to three variants of mRNA, αPPT-A, βPPT-A and γPPT-A, respectively. The three variants determine the synthesis of SP, through the intermediate production of three variants of protachykinin A (αPT-A, βPT-A and γPT-A), respectively, but NK-A is only formed from βPPT-A and γPPT-A[8,9]. The protachykinins, stored in the inside of the vesicles, reach the nerve terminal by axonal transport, during which their excision is produced by the action of some proteases called convertases to generate the final products, which are released in the synaptic cleft through exocytosis, due to its great hydrophilia[1]. NK-B is originated from the pre-protachykinin B gene, by a similar mechanism[8,9]. SP is the most abundant and most extensively studied neuropeptide, both in the CNS and in the autonomic nervous system. The greatest density of SP is located in the dorsal horn of the spinal cord, in the substantia negra, the amygdala, locus ceruleus, hypothalamus and peduncular nuclei, while other cerebral areas have more moderate density (nucleus accumbens, putamen, etc.) or limited one, as in the case of the cortex, hippocampus or cerebellum. Furthermore, SP is expressed in the peripheral nervous system, mainly in the spinal ganglia and vegetative nerves. Three NK receptors, named NK_1, NK_2 and NK_3, have been identified and the existence of a fourth one (NK_4) has been proposed, although it has not been demonstrated. Although all NK share some affinity for them, SP has the greatest affinity for NK_1 receptor and is thus its natural ligand, while NK-A and NK-B are the main ligands of NK_2 and NK_3 receptors, respectively. NK_1 receptors contain seven transmembrane domains and are associated to proteins G[7,8]. They are the most abundant and are widely distributed in the CNS neurons and glial cells, as well as in peripheral tissues, including non-nervous cells. Their greatest density is found in the dorsal roots of the spinal cord and caudate-putamen, but not in the substantia negra. NK_3 receptor is also extensively distributed in the CNS, although its expression does not overlap with that of NK_1 receptors, since NK_3 receptors are mainly in the deep cortical layers, in the nigrostriatal pathways and also in the spinal cord[7]. In regards to NK_2 receptor, its expression has not been firmly established in the CNS, but it is known that it is widely distributed in the peripheral tissues and also in the sensorial neurons of the posterior horns of the spinal cord[7]. As NK_1, NK_2 receptor also contains seven transmembrane domains and is also associated to G proteins[7,8]. SP is extensively co-localized with classical neurotransmitters and with other neuropeptides[11], although, on the contrary to the first ones, its receptors are widely distributed in the CNS. However, there are apparent divergences between SP distribution and that of its receptors in the CNS. This could be due to either SP reaches its receptors by diffusion or that it also binds to NK_2 or NK_3 receptors, or, simply, to the inability of the laboratory techniques to demonstrate the real distribution of the receptors[10,12]. SP is located in presynaptic vesicles, both in dendrites as in the neuronal bodies, from which it is rapidly released by a calcium-dependent process after the application of an acute noxious stimulus or due to the stress effect. NK1 receptors are located in the somatodentritic cell membrane but the binding of the SP rapidly produces migration of these inside the cytoplasm. This internalization is reversible in about 30 minutes, the receptors returning to their initial site and is proportional to the intensity of the stressor stimulus applied. On the other hand, repeated or intense stimulation activates relatively distant neurons by a diffusion mechanism[12].

INVOLVEMENT OF NEUROKININS IN THE PRODUCTION OF AFFECTIVE BEHAVIORS

Involvement of NK, and especially SP, in the mechanisms that generate anxiety and depression arises from several facts. In the first place, both SP and its NK_1 receptor are highly expressed in brain regions critical for the regulation of affective behavior and neurochemical responses to stress[10]. Furthermore, in these regions, SP is co-localized with monoaminergic systems, which are involved in the mechanisms that underlie depression and anxiety[13]. Finally, it has been demonstrated in experimental animals that SP is released in the periaqueductal gray matter (PAG) and limbic structures, in response to aversive or noxious stimuli. Thus, an increase in SP in the hippocampus, septum, PAG and ventral tegmental area of rats subjected to unavoidable electrical discharge, to social isolation and to forced immobilization has been observed[14,16]. More recently, it has been shown that exposure of rats to a stress situation produces a lasting release of SP in the medial nucleus of the amygdala, but not in the central nucleus. Furthermore, bilateral microinjection of a NK_1 antagonist in the medial amygdalar nucleus counteracted the anxiogenic effect induced by the stress situation and also antagonized the anxiogenic effects of SP injection in this area. This would confirm that the medial nucleus of the amygdala is a critical cerebral area for the production of emotional responses to stress[17]. All this indicates that SP regulates the responses to noxious and stress stimuli, participating in the appearance of defensive behavior[13] and, as has also been demonstrated, in cardiovascular reactions to stress[18]. Some studies have also demonstrated the involvement of NK_1 receptor in the genesis of affective behaviors, as response to stress. Thus, maternal separation of guinea pigs pups or stress induced by immobilization in gerbils, produce endocytosis of this receptor in the neurons of basolateral nucleus of the amygdala, which can be antagonized by the systemic administration of NK_1 antagonists[13,19]. Several studies have also investigated the role played in humans by SP in response to stress[20,22]. Some of these studies have also investigated SP function as mediator in stress-induced immune responses, showing a connection between SP in the peripheral nervous system and the immune system[22]. Thus, in a study in patients with chronic pain syndrome, Almay et al.[20] observed an inverse relationship between SP levels in cerebrospinal fluid and subjective perception of «psychic anxiety», evaluated by a visual analogue scale. This correlation was also observed between SP levels and «internal tension» personality trait, determined by the Karolinska Institute personality scales. In a group of parachutists who jumped for the first time, Schedlowski et al.[21] measured SP plasma levels 2 hours before the jump, immediately after and 1 hour after it. On the contrary to β-endorphin plasma levels, which increased immediately before the jump and then decreased, SP plasma levels did not change at any time. These results oppose the idea that SP is released in the face of a stressing situation. However, the subjects with elevated state-anxiety immediately before the jump, evaluated by the State-Trait-Anxiety Inventory (STAI), had significantly higher SP plasma levels in the 3 measurements than subjects with lower state-anxiety, while the trait-anxiety did not modify SP plasma concentrations. This indicates that SP secretion is influenced by the state-anxiety level during exposure to acute stress. In a study with subjects undergoing a diagnostic procedure (colonoscopy or sigmoidoscopy), Fedher et al.[22] found similar results: individuals with a greater anxiety level prior to the examination (assessed by the Multiple Affect Adjective Checklist-Revised [MAACL-R questionnaire]), had higher SP serum levels than the subjects with a low anxiety level and a high correlation between anxiety scores and initial SP levels was found. Furthermore, subjects with initial high anxiety maintained higher SP levels than subjects with low anxiety, once the examination was completed and 3 days after it although, in the latter measurement, the differences no longer reached statistical significance. This suggests that high anxiety levels are associated with greater SP levels in peripheral blood, which remain elevated for a long time after ending the initial anxious situation. These same authors[22] suggest that elevated levels of SP in anxiety states may influence T lymphocyte-mediated immune response. In this study, subjects with elevated anxiety (and SP) levels have significantly higher CD3+ T lymphocyte blood levels and absolute number and percentage of cytotoxic T lymphocytes (CD8+) in blood in comparison with low anxiety individuals in the assessments before and after the therapeutic procedure. Furthermore, there was a highly positive correlation between the percentage of CD8+ T lymphocytes and SP serum levels in the previous control and in those posterior to the stress situation. Although this type of study does not allow establishing a causal relationship, the authors consider that it is very possible that SP forms a part of a «neurogenic alarm system» that would interrelate the CNS, immune and endocrine systems, in which SP would play a role in lymphocytic mobilization, increasing the number of cytotoxic T lymphocytes.

In regards to depression, direct evidence on the existence of neurokininergic hyperactivity in this disorder is scarce. However, an increase in SP levels in the cerebrospinal fluid of depressed patients has been reported[23], although another later study was unable to replicate this finding[24]. More recently, a statistically significant increase has been observed in SP serum levels of patients with major depression, although only 37% of them had a decrease in SP serum levels (between 15% and 50%) after 4 weeks of antidepressant treatment[25], which correlated with a greater response to treatment. Reduction of NK1 receptors in the orbitofrontal cortex of subjects with major depressive disorder, when compared with normal subjects, has also been demonstrated «post-mortem». This would reflect an attempt for adaptation to the increase of SP availability in depressed individuals[26]. It has also been demonstrated in experimental animals that anxiolytics and some antidepressants produce a decrease in SP brain levels[27]. Furthermore, in an experimental depression model in the rat, it was observed that both SP and NK-A levels were increased in the frontal cor-

tex and decreased in the striatum, in comparison with the controls, and that treatment with lithium abolished these differences[28]. As a whole, all these data suggest that NK, and above all SP, are involved in affective behaviors.

EXPERIMENTAL MANIPULATION OF THE NEUROKININERGIC SYSTEM

For the study of the role played by NK in affective behaviors, pharmacological stimulation of their receptors with neurokininergic agonists as well as its inactivation, either with pharmacological agents (receptor antagonists or cytotoxic substances) or by genetic manipulation (knockout mice) has been used[29]. NK studied most has been SP. The potential antidepressant and anxiolytic effects of NK_1 receptor antagonists have raised great interest in the development of these compounds and, consequently, have promoted intense research by the pharmaceutical industry. However, the study of the possible role of SP in affective disorders has been made difficult for a long time due to technical problems derived from the scarce adequacy of NK_1 antagonists for these purposes[5,29]. The first antagonists synthesized were large peptide molecules, which determined their poor penetration of the blood-brain barrier besides having scarce potency and selectivity. In the beginning of the 1990's, CP96345, the first non-peptide type NK_1 antagonist, a substance with elevated affinity for the human NK_1 receptor, was introduced. However, this and other similar compounds had much less affinity for the rat receptors, which made preclinical assessment of these substances very difficult. Other problems are caused because not all these substances have good bioavailability in the CNS and some of them produce non-specific effects at high doses. This implies that, in some studies, enantiomer pairs are used, one of them active in NK_1 receptor and the other inactive, to thus be able to attribute the possible effects observed to the receptor blockade[5]. Recently, new experimental models have been developed with animals that have NK_1 receptors with a peptide sequence that is more similar to that of the human, as the guinea pig, cat, rabbit, etc. For example, the administration of a NK_1 agonist to gerbils produces a rhythmic tapping of the feet, which is equivalent to the behavior response to an aversive stimulus and can be antagonized with a NK_1 antagonist. Thus, it is a frequently used model for the assessment of the penetration of this type of drugs in the CNS[30]. In addition, NK_1 antagonists with high capacity of penetration of the blood-brain barrier and without non-specific effects at high doses have been introduced[5,29].

Neurokininergic agonist and antagonist activity has been studied both in anxiety and depression models as well as in mixed anxiety/depression models. Several preclinical studies show that central infusion of SP or NK_1 receptor agonists in different animals elicits defensive behaviors, as, for example, a conditioned aversive response[13], aggression[31] or the enhancement of the acoustic startle response[32], as well as cardiovascular changes that mimic those seen in response to a threat[18]. In guinea pigs, administration of SP or a NK_1 agonist produced locomotor activation accompanied by audible vocalizations. It has been demonstrated that this response is mediated by NK_1 receptor since the administration of an antagonist, but not of its inactive enantiomer, inhibited these vocalizations[13,33], which were also inhibited by fluoxetine or imipramine, but not by diazepam. Likewise, stress caused by transitorily separating the pups from their mothers induced similar vocalizations that were also inhibited by the administration of different NK_1 antagonists. In this model, chronic administration of the antidepressants phenelzine, imipramine, fluoxetine or venlafaxine as well as the anxiolytics diazepam, chlordiazepoxide or buspirone also cause a dose-dependent and complete inhibition of vocalizations[13,33]. Similarly, intraventricular injection of SP or NK_1 agonists causes an increase in anxiety in mice submitted to the elevated plus-maze test[34-36]. On the contrary, administration of NK_1 antagonists had similar anxiolytic effects to that produced by diazepam[35] and antagonized the anxiogenic effects of pentylentetrazol[37] and SP[36] in this model. Furthermore, it counteracted the decrease in the number of peripheral lymphocytes that are produced when the animals undergo a stress test[36]. In addition, the administration of several NK_1 antagonists produced anxiolytic effects in the elevated plus-maze test in primates[38], as well as in the social interaction model in rats and gerbils[39-42]. Finally, NK_1 antagonists have also demonstrated activity in purer depression models, as that of mild chronic stress in the rat[43]. Regarding NK_2 receptor, both the intraventricular injection of NK-A and of a NK_2 agonist had an anxiogenic effect[35] and inhibited the anxiolytic effect of diazepam[37] in mice, in the elevated plus-maze test. On the contrary, administration of NK_2 antagonists had an anxiolytic effect similar to that produced by diazepam in that model[35] and also produced an inhibition of the anxiogenic action of pentylentetrazol, which was not neutralized by the administration of flumazenil[37]. Other experimental models of anxiety, such as the mouse light/dark box test[44,45], or the human intruder threat model in primates[44], have also served to demonstrate the anxiolytic activity of NK_2 antagonists. Finally, in the mice elevated plus-maze model, administration of NK_3 agonists produced anxiolytic effects[46,47], which were enhanced by pre-treatment with naloxone[47], while the administration of a NK_3 antagonist caused either contrary effects[37,46] or no effect[47].

In summary, experimental evidence suggests that NK_1 or NK_2 agonists would be anxiogenic and NK_1 or NK_2 antagonists are anxiolytics, while the contrary occurs with NK_3 agonists and antagonists, respectively. However, things are not so easy, since it has been seen that NK activity is not uniform, since it depends on the administration site and dose as well as on the model, strain and gender of the animals used[40,48,49]. For example, systemic injection of SP has an anxiolytic effect in the rat when administered at low doses (50 µg/kg), while it produces anxiogenic effects if administered at high doses (500 µg/kg)[50]. In addition, when administered directly in the ventral pallidum, it produces anxiolytic

effects at low doses (1 ng), which are not observed with higher doses (100 ng)[50,52,53]. On the contrary, intraventricular injection of low (1 pmol) or intermediate doses (210 pmol) produces aversive responses that do not appear with higher doses (100 pmol), which tend to be anxiolytic[35,51]. On the other hand, when applied in the dorsal PAG[54], lateral septal nucleus[51] or medial nucleus of the amygdala (but not in the central nucleus)[17], it causes an increase in anxiety. Furthermore, differences have been found in the anxiolytic activity of NK_1 antagonists based on animal gender and experimental model used. Thus, the administration of a NK_1 antagonist had an anxiolytic effect in male rats, in the open field model, but did not have this same effect in female or male rats on the elevated plus-maze test. In addition, differences were recorded in anxiolytic activity based on the rat strain used for the trial[49]. It has also been suggested that SP could be inactive in the CNS, its effects being due to the amino (N)-terminal (SP1-7) and carboxy(C)-terminal (SP5-11, SP6-11 or SP7-11) peptide fragments that are produced by enzymatic metabolism of SP[52,54]. These fragments also have different activities, according to the site of administration in the rat CNS. Thus, it has been observed that injection of the N-terminal fragment in the ventral pallidum produces anxiolytic and pro-mnestic effects[52,53], which are not observed (at least with the same clarity) when it is injected in the PAG[48,54]. Administration of the C-terminal fragment in the ventral pallidum also produces anxiolytic effects and reinforcing properties[52,53], but it showed anxiogenic activity when the administration site was PAG[48,54]. The anxiolytic effect of systemic administration of the N-terminal fragment of SP has also been demonstrated in primates[55].

In addition to the role played by SP in responses to stress (state-anxiety), other studies suggest that SP may also be involved in the genetic mechanisms that predispose to trait-anxiety. Thus, Sudakov et al.[56] measured SP levels in the hippocampus, hypothalamus and middle brain of rats belonging to two strains, one of them with high genetic predisposition to display anxiety (Fischer-344 [F-344/N]) and the other with less emotionality (Wistar Albino Glaxo [WAG/G]) in which greater density of cortical benzodiazepine receptors has been demonstrated previously. As expected, it was demonstrated that anxiety in the face of a new setting evaluated in 5 experimental paradigms (elevated plus-maze test, Vogel lick suppression test, open field conflict, white and black box, and hole board test) was significantly greater in F344/N rats than in WAG/G rats. On the other hand, SP levels at baseline were lower in the rat strain with high anxiety level in comparison with those with low anxiety. Furthermore, SP levels after stress decreased, but only in less anxious rats. All this indicates that the rats genetically predisposed to anxiety not only had less density of benzodiazepine receptors in the cortex but also lower SP brain levels. This study also demonstrated a decrease of the diazepam binding inhibitor (DBI) fragment and the presence of elevated levels of neuropeptide Y (NPY) in more anxious rats, which, for the authors, would constitute compensating mechanisms. Finally, intraventricular administration of low SP doses decreased anxiety in both strains, although the more anxious rats were more sensitive to their effects. However, high SP doses did not have anxiolytic effect, which probably reflects the different SP activity based on the already mentioned differences in the dose and the way of administration. In summary, these findings disagree with those of other studies that find an increase of SP as response to stress14-16, as well as the studies that report an anxiogenic effect after administration of SP or NK1 agonists[13,18,31-36]. However, it seems clear that SP is involved in the genetic bases that predispose the subjects to present high levels of anxiety, the differences between the studies possibly being due to technical aspects as well as dose and way of administration of SP or animal strain studied.

A more solid proof of the involvement of SP in the responses to stress, comes from genetic inactivation studies of the SP-receptor NK1 system, carried out in mice with selective deletion of the Tac-1 gene (knockout mice Tac-1 *[Tac1$^{-/-}$]*)[57]. This gene determines the production of three precursor proteins in mice that give rise, in turn, to several peptides, among them SP and NK-A. In this way, the mutant animals are not capable of producing SP or NK-A and are less sensitive to pain, although they do not otherwise present other alterations in regards to their development or fertility. When their behavior was studied in experimental anxiety models (elevated plus-maze, social interaction, open field, etc.) normal mice *(Tac1$^{+/+}$)* showed, in fact, anxiety when undergoing the tests while these did not seem to be anxiogenic for the mutant animals *(Tac1$^{-/-}$)*. In this same experiment, *Tac1$^{-/-}$* mice subjected to two experimental depression models, such as the forced swimming test (Porsolt test) or tail suspension test, showed a decrease of immobility time when compared with *Tac1$^{+/+}$* mice, similar to that observed in wild-type mice, after treatment with antidepressants: imipramine, amitryptiline or fluoxetine. In addition, in another experimental paradigm of depression, *Tac1$^{-/-}$* mice did not show the hyperactivity produced by bulbectomy that did appear in normal bulbectomized mice. In summary, these findings show that the net effect of the suppression of SP and NK-A production in these animals is the decrease of emotionality, which suggests that the SP and NK-A play a fundamental role in the mechanisms that generate anxiety and depression.

Other authors have also used transgenic mice to show the involvement of NK_1 receptors in affective behaviors[29,31,33,58]. Thus, Rupniak et al.[33], in homozygous mutant mice pups with genetic inactivation (knockout) of the NK_1 receptor *(NK1$^{-/-}$)*, observed a reduction of ultrasonic vocalizations induced when separating the pups from their mothers. Santarelli et al.[29,58] used the same model to study behavior in different anxiety and depression models, such as the elevated plus-maze test, novelty suppressed-feeding or the ultrasonic vocalization test of pups induced by separation from their mothers. These authors also produced pharmacological inactivation of NK_1 receptor by the administration of RP67580,

a mouse and rat-selective antagonist of this receptor. In addition, to monitor the possible non-specific effects that could be produced by the administration of RP67580, one group of animals received RP68651, its pharmacologically inactive enantiomer. In summary, $NK_1^{-/-}$ animals showed a reduction in anxiety in response to stress produced by exposure to the elevated plus-maze test in comparison with wild-type mice $(NK_1^{+/+})$ and with the heterocygote mutants $(NK_1^{+/-})$. Furthermore, in this paradigm, $NK_1^{-/-}$ mice showed the suppression of cortisol increase which did occur in control animals, after the exposure to the test. Likewise, the homocygote mutants had a reduction in anxiety and stress-related responses in comparison with the controls in the other models mentioned. Similarly, normal mice treated with RP67580 manifested a less anxious behavior in response to stress than normal mice treated with placebo. Administration of inactive enantiomer did not show efficacy, so that the effects of RP67580 should be attributed to the antagonism of NK_1 receptors. It is important to stress that the effect of administration of RP67580 was similar to that obtained by administration of diazepam, used for the validation of the experimental models as anxiety ones. On the other hand, the anxiolytic effect of diazepam was not only manifested in normal animals but also in mutant mice $(NK_1^{-/-})$. This indicates that the anxiolytic action of the gabaergic system does not necessarily need the peptidergic system and that, therefore, these two systems may act independently. These same authors, Santarelli et al.[29], determined the induction of the expression of the protein c-Fos, as a measure of the neuronal activation after exposing mice to a stress situation, such as the elevated plus-maze test. On the contrary to normal mice treated with placebo, animals pretreated with RP67580 and NK_1 knockout mice had significantly lower induction of c-Fos protein in the paraventricular nucleus of the hypothalamus as response to stress, which is consistent with the fundamental role that this nucleus plays in the normal response to stress. These findings have been partially replicated by suppression of NK_1 receptor through the administration of the neurotoxin SP-Saporin (SP-SAP). SP-SAP is the conjugation of saporin (a cytotoxic agent that is a protein synthesis inhibitor in the ribosomes, incapable of crossing the blood-brain barrier) with the SP. In this way, administration of SP-SAP uses the internalization of NK_1 receptors to produce selective death of the neurons that express this receptor. By injection of SP-SAP in the rat's amygdala, a reduction of anxiety has been demonstrated in the elevated plus-maze test in comparison with control animals[12]. However, the contrary has also been demonstrated, that is, the anxiogenic activity of the intraamygdalar administration of SP-SAP in mice[59]. For these authors[59], the discrepancy between their results and those obtained in $NK_1^{-/-}$ animals may be due to the different animal species studied or more probably, to the different techniques used. In fact, administration of SP-SAP not only causes the elimination of NK_1 receptors, as occurs with the suppression of the receptors by administration of antagonists or genetic means, but also causes necrosis of the neuron and, with this, the loss of the receptors and co-expressed neurotransmitters, as well as the interneuronal connections.

Interaction of SP with other neurotransmission systems

It has been speculated that the effects of NK_1 antagonists on anxiety and depression could involve mechanisms of action independent from the noradrenergic and serotonergic pathways[13]. However, it is known that SP is co-localized extensively with classical neurotransmitters and with other neuropeptides[11,60]. It has also been demonstrated in primates and humans that SP and serotonin are co-expressed in approximately 50% of the dorsal raphe nucleus (DRN) neurons[60]. These data, together with other findings as, for example, the decrease in SP levels in the rat forebrain after chronic treatment with antidepressants, makes it unlikely that the monoaminergic systems do not contribute to the effects of NK_1 antagonists[61]. Since SP-containing neurons are co-localized, among others, with serotonergic and noradrenergic neurons and considering the involvement of these two systems in the processes that underlie anxiety and depression, the relationship existing between them and the SP-NK_1 system has been investigated. Thus, it has been observed that acute or chronic administration of L-760735 (a NK_1 antagonist) causes an increase in the activity of DRN serotonergic neurons, producing an increase in the firing rate, which is accompanied by a change in the neuronal firing pattern to a more effective one. These data suggest that endogenous SP participates in the functional inhibition of this nucleus[62]. In the previously mentioned study of Santarelli et al.[58], the authors studied in vivo the activity of DRN serotonergic neurons in normal mice, treated or not with a NK_1 antagonist (RP67580), as well as in $NK_1^{-/-}$ mutant mice. These investigators observed that DRN neurons, both for NK_1 knockout mice and in normal mice treated with the NK_1 antagonist, showed an increase in their firing rate, in comparison with the untreated normal animals. This indicates that serotonergic activity of DRN is inhibited by the SP-NK system. The increase, at more than double the firing rate of the serotonergic neurons, was seen after 30 minutes of the administration of the NK_1 antagonist. This contrasts with the increase in the serotonergic activity that is also produced by most of the antidepressants, since this is observed only after chronic treatment. Thus, it was investigated if the activity of 5-HT_{1A} autoreceptors is modified in these circumstances, to allow for such a rapid increase of the serotonergic activation. Thus, a reduction in the number of 5-HT_{1A} receptors was observed in the DRN of NK_1 Knockout mice[63]. Furthermore, the administration of 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT), a selective serotonergic agonist of the 5-HT_{1A} receptors, directly in the DRN, caused a dose-dependent inhibition of the neuronal firing rate in normal mice (as was expected), but not in $NK_1^{-/-}$ mice[58,61] or in normal rats pretreated with a NK_1 antagonist[64]. This indicates that SP decreases the expression of presynaptic 5-HT_{1A} receptor. On the contrary, the applica-

tion of 8-OH-DPAT in the hippocampus, where postsynaptic 5-HT_{1A} receptors of DRN neurons are expressed, caused an inhibition of pyramidal neuron firing both in *$NK_1^{-/-}$* animals as well as in normal ones treated or not with NK_1 antagonist, indicating that SP does not change the activity of the postsynaptic 5-HT_{1A} receptors[58,61]. Similarly, long term treatment with CP-96,345, a NK_1 antagonist, caused significant desensitization of presynaptic 5-HT_{1A} autoreceptors, accompanied by a 50 % and 90% increase in the firing rate of DRN neurons at 2 and 14 days of treatment, respectively. Activation of postsynaptic 5-HT_{1A} receptors of pyramidal neurons of the hippocampus was also observed after 14 days of treatment[64], as occurs with the antidepressants in this same model[61] and in agreement with the latency time observed in the clinics until the appearance of these drugs' effects. On the other hand, it has been demonstrated that the in vitro administration of NK_1 antagonists has no effects on the sensitivity of the presynaptic 5-HT_{1B} autoreceptors or on the postsynaptic α_2 receptors located in the serotonergic terminals, which regulate serotonin release by an inhibitory action on it[61]. In summary, these findings suggest that the inactivation of the SP-NK_1 system causes an increase in the firing rate of the dorsal raphe nucleus neurons, through the reduction of the expression of presynaptic 5-HT_{1A} autoreceptors (downregulation). In turn, this increase of the raphe activity stimulates 5-HT_{1A} receptors of the hippocampus (postsynaptic), whose function is preserved. This is consistent with the lower anxiety showed by NK1 knockout mice, although in another study with normal guinea pigs[62] treatment with a NK_1 antagonist did not cause desensitization of 5-HT autoreceptors. In the opinion of this study's authors[62], this difference could be a reflection of an adaptation more than a real desensitization of the receptors, which occurred in the transgenic mice.

As can be seen, there is a close relationship between the SP-NK_1 system and serotonergic pathways. However, using immunocytochemical techniques, it was demonstrated that, in spite of the abundance of NK_1 receptors in DRN (especially in the dendrites of the dorsomedial subregion), the overlapping between these receptors and serotonergic neurons is quite discreet, both in the rat[65,66] and in the mouse[29,58]. In addition, it has been demonstrated that a large part of DRN and PAG NK_1 receptor-expressing neurons are glutamatergic or, in a smaller proportion, encephalinergic. Consequently, if most of NK_1 receptors are not located in serotonergic neurons, the modulation of serotonin activity by NK_1 receptors should be indirect, through other mechanisms. Thus, it is reasonable to think that SP would act primarily on the mentioned systems (mainly on the glutamatergic) in the DRN and the PAG and these, in turn, would do it on the monaminergic systems[65]. In agreement with this hypothesis, it has been demonstrated by the intracellular recording of the *in vitro* electrical activity that the activation of the firing of raphe serotonergic neurons produced by the direct application of SP is blocked by the administration of tetrodotoxin. This indicates that this activation is the result, in turn, of the direct activating action of the glutamatergic interneurons on the serotonergic neurons[67].

Since noradrenergic neurons of the locus ceruleus stimulate serotonergic neurons of the raphe, the possible location of NK_1 receptors in the locus ceruleus was also investigated and the existence of a high density of NK_1 receptors in noradrenergic neurons of this nucleus was demonstrated[29,58,61]. It has also been shown that the application of SP on noradrenergic neurons causes an excitatory effect[61]. On the other hand, NK_1 knockout mice do not show an increased spontaneous firing rate of noradrenergic neurons[61], and this is also not observed after administration of some NK_1 antagonists to rats or gerbils, although it has been observed in mice treated with RP68750, which is probably due to a specific effect of this substance[61]. However, administration of a NK_1 antagonist does produce attenuation of the inhibition of noradrenergic and serotonergic activity produced by the administration of α_2-adrenergic agonist clonidine [61,68]. All this suggests that NK_1-receptor antagonists activity would be produced through the noradrenergic neurons of the locus ceruleus, which would, in turn, stimulate serotonergic neurons of the DRN. The demonstration that integrity of the noradrenergic neurons is necessary to produce serotonergic activation after the administration of a NK_1 antagonist supports this hypothesis. In fact, in mice treated with RP68750, there is an increase in the activity of serotonergic and noradrenergic neurons few minutes after the drug administration. However, the activating effect on serotonergic firing did not occur in rats pretreated with the selective noradrenergic neurotoxin DSP-461. In this sense, NK_1 antagonists are differentiated from the SSRIs, which do not increase the firing rate in the DRN and would be similar to mirtazapine and bupropion, two dual action-mechanism drugs, which does have this action, as long as the integrity of the noradrenergic pathways is maintained[61]. These findings also contradict the previously explained notion[13] that the effects (above all the anxiolytic ones) produced by the SP-NK_1 system are independent of the serotonergic system, as had been suggested when observing that the effects of NK_1 antagonists appear rapidly, without the latency period that is observed with serotonergic drugs.

Besides the glutamatergic and noradrenergic systems, other neurotransmission systems may also be involved in the stimulation of DRN neurons produced by NK_1 antagonists. On the contrary to the rat and mouse, guinea pigs and primates have relatively few NK_1 receptors in the DRN, which indicates that the SP antagonists probably owe their effects to the blockade of these receptors in other brain areas[62]. However, in humans and primates, high density of NK_1 receptors has been found in the lateral habenula, a structure from which the main descending projections (gabaergic) from the forebrain to the DRN originate which maintain a functional inhibitory tone on the latter. In turn, the DRN sends ascending projections to brain structures that also have a high density of NK_1 receptors and are involved in the response to stress, as the amygdala and cingulate cortex.

The latter send efferences to the lateral habenula, thus closing a circuit that would be involved in the coordination of the responses to stress[62]. In support of this hypothesis, direct application of a NK_1 antagonist in the lateral habenula caused an increase of the DRN firing rate while neither the application on areas adjacent to the habenula nor the direct application on the DRN modified its activity[62]. Finally, in relationship with other neurotransmitters, there has been speculation on the possible contribution of dopaminergic pathways to the antidepressive effects of NK_1 antagonists. In fact, systemic administration of the NK_1 antagonist GR205,171 to rats produced the activation of the mesocortical dopaminergic pathway, causing an increase in the firing rate of dopaminergic neurons of the ventral tegmental area and of dopamine levels in the frontal cortex, although not in the striate or in the nucleus accumbens, without affecting the activity of DRN neurons[69]. The interaction of NK_1 receptor-expressing neurons with the neurons of the CRF (corticotropin releasing factor) system, which are co-localized with serotonergic neurons of the dorsomedial subregion of the DRN which, in turn, sends projections to limbic areas, has also been described[66].

Apart from that explained, it is clear that the complex relationship of SP with other neurotransmitter systems has consequences that go beyond the production of affective behaviors. For example, the existence of a close connection between dopaminergic pathways and SP in the nigrostriatal system[52] and the mesolimbic pathway[70], as well as the involvement of SP in the reward pathways is known[52,59]. It is also known that there are NK_1 receptors in the cholinergic ventral pallidal neurons (the main source of acetylcholine in the cortex), which stimulate these neurons, and it has been demonstrated that the administration of SP in this area causes an increase of acetylcholine in the frontal cortex and of dopamine in the nucleus accumbens[52]. Finally, the extensive co-location of the neurokininergic systems with other neuronal systems allows NK to also participate in the mechanism underlying a large number of functions not related with affective behaviors, as, for example, pain, memory, learning, or functional recovery after a nervous lesion, whose study is beyond the scope of this paper.

CLINICAL TRIALS WITH NK_1 ANTAGONISTS

Kramer et al.[13] reported for the first time the efficacy of MK-869 (also called aprepitant), a NK1 antagonist without affinity for serotonergic, dopaminergic or noradrenergic receptors, in the treatment of depression. A randomized phase II, 6 week, comparative study with paroxetine and placebo was carried out. The patients were diagnosed of major depression, single or recurrent episode, according to DSM-IV criteria and must score 22 or more on the Hamilton depression rating scale (HAM-D17). Furthermore, they should have moderately high anxiety, determined by a score on the Hamilton anxiety rating scale (HAM-A) equal to or greater than 15, and a score at least 4 (moderately ill) on the severity subscale of the Clinical Global Impression scale (CGI-G). After 6 weeks of treatment with 20 mg/day of paroxetine, 300 mg/day of aprepitant or placebo, there was a similar improvement in the mean score of the HAM-D21 (main efficacy endpoint) in both active treatment groups that was statistically superior to that recorded in the placebo group. In addition, on the contrary to that observed in the paroxetine treated group, treatment with aprepitant was accompanied by a progressive reduction in the HAM-A score, which reached statistical significance at weeks 4 and 6. Tolerability in the aprepitant group was similar to that of the placebo and superior to that of paroxetine, group in which there were more drop-outs due to adverse effects. Although these results strongly suggest an antidepressive and anxiolytic effect of NK_1 antagonists, a later dose-finding study, comparative with placebo and fluoxetine, could not demonstrate differences among the three treatment arms and finally the development of aprepitant as antidepressant or anxiolytic was discontinued[71]. This also occurred with other similar drugs[71,72], with NK_1 antagonist activity, whose development has been discontinued.

CONCLUSIONS

SP is the most abundant and best studied neuropeptide, both in the CNS and peripheral nervous system. It is involved in the neural mechanisms that modulate affective behavior, memory, reward and neuronal repair and has been involved in the pathogenesis of different psychiatric and non-psychiatric diseases. Since the discovery of SP, NK have been the object of constant research and the advances in the knowledge of its physiological function and also in pathological conditions have been incessant. However, the reality is that, in spite of the important advances made in the last 50 years, and although the NK_1 receptor has been a pharmacological target pursued since 20 or 30 years ago, the clinical applications derived from these progresses have been very scarce.

SP is extensively expressed in the brain circuits that mediate the behavior and affective responses to stress and seem to be involved in the production of these responses. Although its action is complex and poorly known, it is believed that it produces its effects indirectly, through the modulation of the monoaminergic activity and that of other neurotransmission systems. NK_1-receptor antagonists have shown preclinical activity in different anxiety and depression models, thus providing a theoretical basis for the use of these drugs in the treatment of these diseases. In humans, NK_1 antagonists seem to behave as antidepressants and also lead to an improvement of anxiety associated to depression, with a similar tolerability to that of the placebo and absence of the side effects characteristic of serotonin re-uptake inhibitors. However, it is surprising that, in spite of the intense research effort performed in this field, only 3 positive studies have been published with NK_1 antagonists[13,61,71,72] and that none of these drugs have reached the market, sin-

ce their initial efficacy could not be replicated. However, other NK receptor antagonists continue to be developed and it cannot be ruled out that they may be eventually used in the treatment of anxiety and/or depression in the future.

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