Levels of Neurokinin A, Neurokinin B and Substance P in Rabbit Iris Sphincter Muscle†

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Abstract—We investigated the contents of neurokinin A, neurokinin B and substance P in the rabbit iris sphincter muscle, combining HPLC and radioimmunoassay, as our previous reports indicated that a slow component of neurogenic contractions of this muscle is most probably mediated by such tachykinins. The concentrations of these tachykinins were 44.3±8.7 fmol/mg protein, 35.3±10.2 fmol/mg protein and 186.8±29.8 fmol/mg protein (N=4), respectively. These results demonstrated that neurokinin A, neurokinin B and substance P are all present in the rabbit iris sphincter muscle.

Substance P has been thought to be an excitatory neurotransmitter which functions in the spinal cord and in other areas of the nervous system (1). In addition to substance P, two novel tachykinins have recently been identified in mammals. Neurokinin A (also called substance K, neuromedin L) (2-4) and neurokinin B (also called neuromedin K) (2, 5) have been isolated from spinal cord. The rabbit iris sphincter muscle, which is highly sensitive to tachykinin, is innervated by the substance P-immunoreactive, trigeminal nerve (6), the stimulation of which causes a noncholinergic, nonadrenergic contraction (7, 8). Since this noncholinergic, nonadrenergic response was inhibited by a substance P antagonist, we considered its possible mediation by substance P or substance P-like peptide(s) released from the trigeminal nerve (9, 10). Recently, we have compared responses to substance P and to neurokinin A and attempted to determine which of these tachykinins is most involved in the substance P-immunoreactive nerve-mediated response in the rabbit iris sphincter muscle. These studies have suggested that noncholinergic, nonadrenergic contraction induced by electrical transmural stimulation is predominantly mediated by neurokinin A rather than by substance P, even though both these tachykinins are released concomitantly from the trigeminal nerve (11).

The presence of substance P in rabbit iris-ciliary body rings has been reported (12), but there has been no report on the existence of neurokinin A in the iris sphincter muscle. In the present study, we examined the contents of neurokinin A, substance P and related peptide, neurokinin B, in rabbit iris sphincter muscle, combining HPLC and radioimmunoassay.

Albino rabbits of either sex and weighing 2 to 3 kg were anesthetized with sodium pentobarbital (20 mg/kg, i.v.) and exsanguinated. The eyes were removed within 15 min after death. One strip of iris sphincter muscle was cut from each eye. Tachykinins were measured according to the method of Kanazawa et al. (13). In brief, the two strips from each rabbit were pooled and homogenized in a glass homogenizer in 3 ml of 1 N acetic acid containing 10 mM HCl, 0.1% β-
mercaptoethanol and 0.1% trifluoroacetic acid (TFA). After centrifugation, the supernatant was injected into Sep-pak C18 and washed twice with 5 ml of 10% acetic acid. The peptide fraction was eluted with 0.1% TFA containing 50% acetonitrile. The separation of tachykinins prior to radioimmunoassay was performed by HPLC. Eluates from Sep-pak were diluted with 0.1% TFA and injected onto an Ultrasphere-ODS (4.6×250 mm) column pre-equilibrated with 0.1% TFA. Elution was initiated with a solution of 0.1% TFA+10% acetonitrile. The concentration of acetonitrile was linearly increased from 10% to 50% in 30 min and kept at 50% for another 10 min. The flow rate was 1 ml/min: each tube collected 0.5 ml of eluate. Neurokinin A was always eluted at fractions 43–45, substance P at 51–53, and neurokinin B at 55–57. Aliquots from each tube were evaporated to dryness in a freezing vacuum and processed for radioimmunoassay. 8Tyr-substance P and Bolton-Hunter neurokinin A were radio-iodinated with 125I-Na by the chloramine-T method (14). The labelled compounds were applied to Sep-pak C18 and eluted with a solution of 10% acetic acid containing acetonitrile. The radioimmunoassay system for substance P was the same as described previously (14). The assay system for neurokinin A and B was essentially the same as that for substance P. Assay buffer was prepared as 50 mM barbitone buffer (pH 8.6) containing 5% human plasma and 0.1% β-mercaptoethanol. Standard neurokinins or unknown sample (dissolved in 300 μl of assay buffer) were incubated with anti-neurokinin A antiserum (final dilution of 1:150,000) at 4°C for 4 hr. Then, 125I-labelled Bolton-Hunter neurokinin A (100 μl in assay buffer) was added and further incubated at 4°C for 18–20 hr. One ml of Dextran T-70-coated charcoal suspension in assay buffer without human plasma and β-mercaptoethanol was added. After 30 min standing, the mixture was centrifuged at 3000 rpm for 10 min at 4°C. The supernatant and precipitate were separated by decantation and counted. The same procedures were applied for measurement of neurokinin B except using the standard curve for neurokinin B instead of that of neurokinin A. Since it is difficult to obtain highly specific antiserum against neurokinin A or neurokinin B, in the present study, the polyclonal antiserum against neurokinin A which can also cross-react 25% with neurokinin B was used in the radioimmunoassay system following the separation of these tachykinins by HPLC. Protein content was determined by the method of Lowry et al. (15).

The contents of neurokinin A, neurokinin B and substance P in the rabbit iris sphincter muscle are shown in Table 1. The content of substance P was higher than that of neurokinin A and neurokinin B. The contents of neurokinin A and B were almost the same. It has been reported that the substance P level of normal rabbit iris-cilliary body preparation was 414 pg/one preparation (12). According to the dimensional change, 186.8 fmol/mg protein would be equivalent to 534 pg/one iris sphincter muscle in this study. This value is close to the 414 pg/one iriscilliary body preparation.

The contents of substance P and neurokinin A in the iris sphincter muscle and in rat cerebral cortex (13) were similar. It is also noted that the ratio of substance P to neurokinin A and the ratio of substance P to neurokinin B in the iris sphincter muscle were similar, although it has previously been reported that the ratio of substance P to neurokinin A was much higher than that of substance P to neurokinin B in rat brain and

<table>
<thead>
<tr>
<th>Substance P</th>
<th>Neurokinin A</th>
<th>Neurokinin B</th>
<th>SP</th>
<th>SP</th>
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<tbody>
<tr>
<td>(SP)</td>
<td>(NKA)</td>
<td>(NKB)</td>
<td>NKA</td>
<td>NKB</td>
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<tr>
<td>186.8±29.8</td>
<td>44.3±8.7</td>
<td>35.3±10.2</td>
<td>4.2</td>
<td>5.3</td>
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<tr>
<td>19.8±2.9</td>
<td>4.6±0.8</td>
<td>3.7±0.9</td>
<td>4.3</td>
<td>5.4</td>
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Each value is the mean±S.E. of four rabbits.
spinal cord (13, 16) with the exception of the cerebellum (13) and the ventral part of the lumbar cord (17). Therefore, it is interesting to note that the ratio of neurokinin A to neurokinin B was almost 1:1 in the rabbit iris sphincter muscle.

It is important to consider the results of Nawa et al. (18) which suggest the existence of two types of preprotachykinin mRNAs in bovine brain. They showed that β-preprotachykinin mRNA contains not only the substance P sequence but also the neurokinin A sequence, whereas α-preprotachykinin mRNA lacks the latter sequence, containing only the one for substance P. The same group also reported that there are regional differences in the distribution of the relative levels of preprotachykinin mRNAs in the bovine brain and that there are α- and β-preprotachykinin mRNAs in the trigeminal ganglion (19). These reports suggests that substance P and neurokinin A are released from trigeminal nerve terminals since the rabbit iris sphincter muscle is considered to be innervated by the trigeminal nerve (6, 9). Indeed, we found substance P and neurokinin A in the rabbit iris sphincter muscle in this study. As substance P and neurokinin A exhibit markedly different biological effects on the rabbit iris sphincter muscle (11), such differences may be attributed to the existence of three types of tachykinin receptors, termed SP-P, SP-E and SP-K receptors (20).

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