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Short communication

EFFECTS OF SUBSTANCE P-(1-9) NONAPEPTIDE AMIDE ON INACTIVATION OF SUBSTANCE P IN VITRO

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The effects of substance P nonapeptide amide on inactivation of substance P by the guinea-pig ileum and urinary bladder preparations and of substance P hexapeptide amide, bradykinin and acetylcholine by the guinea-pig urinary bladder, have been investigated. Significant inhibition of inactivation of Substance P was found only in the urinary bladder. None of the other agents were significantly affected. These results suggest that the previously reported agonist effects of substance P nonapeptide may be due to inhibition of inactivation of SP in vivo and this effect is possibly specific.

Substance P Substance P-(1-9) Guinea-pig ileum Guinea-pig urinary bladder Degradation

1. Introduction

The biological activity of the undecapeptide substance P (SP) is generally thought to reside in the C-terminal hexapeptide region of the molecule (Niedrich et al., 1981; Bury and Mashford, 1977) since N-terminal fragments show no agonist or antagonist activity in classical biological assays (Chipkin et al., 1979; Piercey and Einspahr, 1980). However, we have reported that iontophoretic application of substance P-(1-9) nonapeptide amide (SP-(1-9)) onto neurones in the substantia nigra, or its microinjection into the ventral tegmental region, resulted in effects similar to those produced by SP (Growcott et al., 1981). Similarly, Piercey and Einspahr (1980) have demonstrated that iontophoretic application of SP-(1-10) amide onto cat dorsal horn neurones caused excitation. Both groups of workers confirmed the lack of activity of these analogues in the guinea-pig isolated ileum assay, whereas SP and C-terminal fragments produced potent contractile effects. Their conclusion was that different receptors could exist for SP in the CNS and periphery.

An alternative explanation was proposed by Briggs et al. (1981), following their studies with SP-(1-9) and SP on the neonatal rat hemicord. They suggested that the activity obtained with SP-(1-9) might be due to displacement of endogenous SP from binding sites in the tissue. However, an alternative possibility could be that SP-(1-9) interferes with the breakdown of SP in the systems which had been used. We have therefore investigated the effects of SP-(1-9) on SP inactivation by guinea-pig ileum and urinary bladder preparations in vitro.

2. Materials and methods

Female guinea pigs (Duncan-Hartley, 300-350 g) were killed by cervical dislocation and the urinary bladder and terminal ileum removed. Strips of bladder and segments of ileum were suspended in 5 ml organ baths containing Krebs solution at 37°C and gassed with 95% oxygen and 5% carbon dioxide. To determine the time course of inactivation of SP, a submaximal concentration of the peptide was added to baths containing either ileum or bladder and left in contact with the tissues for

30 min. Aliquots of bath fluid were taken at times 1, 3, 6, 12, 20, 25 and 30 min and assayed for contractile activity on a second guinea-pig ileum preparation. Plotting $\log_{10}$ percentage response against time gave a straight line relationship. Thus it was assumed that inactivation of SP followed 1st order kinetics and therefore the results were calculated as the theoretical half-life ($t_{1/2}$). To investigate the effects of SP-(1-9) on the inactivation of SP, the experiments were repeated following prior incubation of either tissue with SP-(1-9) 10 μM for 5 min. To check the specificity of effect of SP-(1-9) on inactivation processes, its effect on acetylcholine, bradykinin and substance P-(6-11) hexapeptide amide SP-(6-11)-induced responses were also investigated in the urinary bladder.

The following drugs and peptides were used: synthetic SP (Sigma), SP-(6-11) amide (Peninsula), SP-(1-9) (synthesised by Mr. N.N. Petter, ICI), acetylcholine chloride (Sigma), bradykinin triacetate (Sigma).

3. Results

Our results demonstrate that both ileum and urinary bladder inactive SP and that this occurs significantly faster in the ileum (fig. 1). Moreover, the urinary bladder inactivates SP and SP-(6-11) at similar rates whereas bradykinin is inactivated more slowly than SP (figs. 1 and 2). Acetylcholine was inactivated extremely rapidly i.e. responses fell to 20% of the initial maximum within 20–30 s,

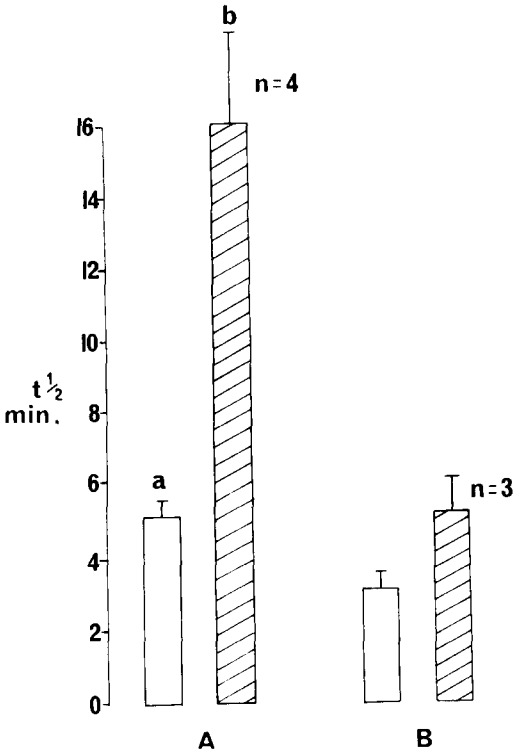

Fig. 1. (A) The effect of substance P-(1-9) on the half-life of substance P in the guinea-pig urinary bladder. □ Substance P; ▨ substance P in the presence of substance P-(1-9) 10 μM, 5 min. (B) The effect of substance P-(1-9) on the half-life of substance P in the guinea-pig ileum. □ Substance P; ▨ substance P in the presence of substance P-(1-9) 10 μM, 5 min. Vertical bars are S.E.M. [a] Significantly different from 'ileum', $P<0.02$, using Student's non-paired t-test. [b] Significantly different from SP alone, $P<0.01$, using Student's non-paired t-test.

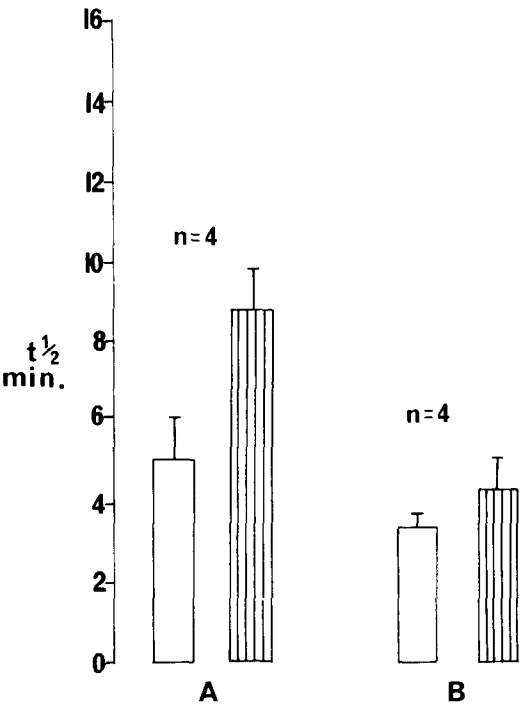

Fig. 2. (A) The effect of substance P-(1-9) on the half-life of substance P-(6-11) in the guinea-pig urinary bladder. □ Substance P-(6-11); ▥ substance P-(6-11) in the presence of substance P-(1-9) 10 μM, 5 min. (B) The effect of substance P-(1-9) on the half-life of bradykinin in the guinea-pig urinary bladder. □ Bradykinin; ▥ bradykinin in the presence of substance P-(1-9) (10 μM, 5 min. Vertical bars are S.E.M.

making it extremely difficult to assay for inactivation with the ileum assay technique.

No changes in responses to acetylcholine in the presence of SP-(1-9) were observed. However, the presence of SP-(1-9) significantly decreased the rate at which SP was inactivated in the bladder only (fig. 1), whereas no significant changes were seen with bradykinin or SP-(6-11) (although there was a trend for the rates to be decreased for these agonists also) (fig. 2).

4. Discussion

The effect of SP-(1-9) on inactivation of SP was probably due to substrate competition for an enzyme or enzymes involved in the degradation of this peptide by the urinary bladder. The fact that SP appears to be so rapidly inactivated may be responsible for the fade of response that is observed in the guinea-pig ileum and urinary bladder following a single application of SP. This has been suggested by Holzer et al. (1981) who reported that degradation, or even uptake into tissue stores, may account for the fade which is characteristic of the response to SP in the ileum. However, Teichberg et al. (1981), suggested that no significant degradation of SP occurs in the urinary bladder since they found that bath fluid containing SP which had been exposed to tissue for 5 min and reapplied to the same tissue 30 min later produced identical responses. One possible explanation could be that degradation of SP to a shorter equiactive sequence occurred during the 5 min incubation or the 30 min period in buffer alone. Degradation would not have been apparent during the 5 min exposure if desensitisation of the tissue had occurred. Furthermore, separation of the bath fluid from the tissue containing the catabolic enzymes is likely to have removed any opportunity for degradation during the 30 min in buffer alone. Thus, further breakdown would not have proceeded. Indeed, we have shown that the tissue must be present for inactivation to occur, indicating that the majority of degradative enzymes do not leach out the tissue into the bathing medium. The effects of SP-(1-9) in the urinary bladder appear to be in good agreement with the findings of Lee et al. (1981) who demonstrated inhibitory effects of SP-(1-9) on degradation of SP by human brain in vitro. We suggest that the agonist-like effects with SP-(1-9) observed by Briggs et al. (1981) and with SP-(1-10) by Piercey and Einspahr (1980) might be due primarily to an inhibitory effect on enzymes that degrade SP, leading to an accumulation of endogenous transmitter to levels sufficient to produce biological activity.

References

Briggs, I., J.W. Growcott, A. Jamieson and M.J. Turnbull, 1981, Depolarizing actions of Substance P 1-9 amide on the isolated spinal cord of the neonatal rat, Br. J. Pharmacol. 74, 294P.

Bury, R.W. and M.L. Mashford, 1977, Substance P: its pharmacology and physiological roles, Aust. J. Exp. Biol. Med. Sci. 55, 671.

Chipkin, R.E., J.M. Stewart, V.E. Sweeney, K. Harris and R. Williams, 1979, In vitro activities of some synthetic Substance P analogues, Arch. Int. Pharmacodyn. Ther. 240, 193.

Growcott, J.W., J.S. Morley, N.N. Petter, R. Pinnock, M.J. Turnbull and G.N. Woodruff, 1981, Difference between central and peripheral actions of Substance P and Substance $P_{(1-9)}$ nonapeptide, Br. J. Pharmacol. 72, 504P.

Holzer, P., P.C. Emson, L.L. Iversen and D.F. Sharman, 1981, Regional differences in the response to Substance P of the longitudinal muscle and the concentration of Substance P in the digestive tract of the guinea pig, Neuroscience 6, 1433.

Lee, C.M., B.E.B. Sandberg, M.R. Hanley and L.L. Iversen, 1981, Purification and characterisation of a membrane bound Substance P degrading enzyme from human brain, European J. Biochem. 114, 315.

Niedrich, H., P. Oehme, B. Mehlis, M. Bienert, J. Bergmann and H. Berger, 1981, Substance P – Structural features and regulatory functions in: Perspectives in Peptide Chemistry (Karger, Basel) p. 344.

Piercey, M.F. and F.J. Einspahr, 1980, Use of Substance P partial fragments to characterise Substance P receptors of cat dorsal horn neurones, Brain Res. 187, 481.

Teichberg, V.I., S. Cohen and S. Blumberg, 1981, Distinct classes of Substance P receptors revealed by a comparison of the activities of Substance P and some of its segments, Reg. Pept. 1, 327.