Presence of NK₃-sensitive neurones in different proportions in the medial habenula of guinea-pig, rat and gerbil

P. Boden & G.N. Woodruff

Parke-Davis Neuroscience Research Centre, Addenbrookes Hospital Site, Hills Road, Cambridge CB2 2QB

Electrophysiological recordings were made from neurones of the medial habenula (Mhb) in brain slices obtained from guinea-pig, rat and gerbil brain. The selective NK₃ agonist, senktide, was used to determine the relative number of NK₃-sensitive-neurones in the Mhb of each species. The proportion of neurones responding to NK₁ (Sar⁹Met(O₂)¹¹SP) and NK₂ (βAla⁸NKA(4–10)) agonists was also assessed. All (65/65) of the guinea-pig Mhb neurones tested were excited by the NK₃ agonist, but NK₁ and NK₂ agonists were without effect. NK₃ responses in the guinea-pig were not altered by the presence of a selective NK₁ antagonist, CP-99,994. NK₁, NK₂ and NK₃ agonists were without effect on Mhb neurones from gerbil brain slices. In agreement with findings from previous studies, a population of rat Mhb neurones responded to NK₁ or NK₃ agonists alone or were excited by both. These data show that there is a difference in both the number of NK-sensitive neurones and the type of NK response found in the medial habenula of the three species. The high sensitivity to an NK₃ agonist, combined with the apparent lack of NK₁ and NK₂ responses in the guinea-pig Mhb makes this preparation ideal for studies of central NK₃-mediated events.

Keywords: NK₃ receptor; medial habenula

Introduction Three types of neurokinin (NK) receptor have been identified in mammalian brain (see review by Guard & Watson, 1991), and functional data exist to support the presence of both NK₁ and NK₃ receptors in differing relative densities throughout various brain regions. In the rat, *in vitro* electrophysiological recordings have demonstrated the presence of NK responses in a sub-population of *substantia nigra* neurones which are excited only by NK₃ agonists (Keegan *et al.*, 1992), whilst in the medial habenula nucleus (Mhb), neurones are found which respond to either, or both, NK₁ and NK₃ agonists (Norris *et al.*, 1993).

The use of selective antagonists for NK₁ receptors has shown that a species difference exists between NK₁ antagonist binding sites in the rat and guinea-pig brain, with the latter being more representative of the human NK₁ receptor (Gitter *et al.*, 1991). There is now evidence to support the same conclusion for central NK₃ receptors (Petitet *et al.*, 1991). To date, although experiments on the expressed human NK₃ receptor suggest an intra-species difference (Buell *et al.*, 1992), confirmation of an inter-species difference between NK₃ receptors awaits the development of selective NK₃ antagonists.

Given the possibility that NK₃ receptors in the guinea-pig brain might mirror more accurately those found in the human brain than the rat brain, the purpose of this study was to investigate the nature and type of NK response found in the Mhb of three species. We chose to use guinea-pig, where information on the nature of NK receptor types in the brain is restricted to the *locus coeruleus* which possesses sub-populations of neurones responding to both NK₁ and NK₃ agonists (Seabrook *et al.*, 1992; McLean, 1993) rat and gerbil. No data are available on functional NK responses in the gerbil brain.

Methods *Brain slice preparation* Coronal slices (350 μm) were prepared from brains of guinea-pig (Dunkin-Hartley, 300–500 g), gerbil (Mongolian, 40–60 g) or rat (Wistar, 150–300 g) brain using a Vibratome (Oxford Instruments). One such slice, cut at the level of the Mhb (bregma – 3.8 to – 4.2 mm) was placed in a perfusion chamber and perfused with artificial cerebrospinal fluid (ACSF) flowing at 4 ml min⁻¹. All experiments were performed at 37°C. Extracellular

recordings were made from individual spontaneously firing neurones in the Mhb. Agonists were applied for 1 min. Slices were pretreated with antagonists for 30 min before addition of agonists in the continued presence of the antagonist. Details of the subsequent data analysis have been reported elsewhere (Boden *et al.*, 1991).

Drugs All NK agonists were obtained from Peninsula Labs. and applied at known concentrations directly dissolved in the ACSF from a 1 mM stock solution in dimethyl sulphoxide (DMSO). The agonists used were; senktide (succinyl-[Asp⁶MePhe⁸]substance P(6–11)); Sar⁹SP(Sar⁹Met(O₂)¹¹substance P); neurokinin B(NKB); β-Ala⁸NKA(4–10); SpOMe (substance P methyl ester). The highly selective NK₁ antagonist, CP-99,994 ((+)-2S,3S)-3-(2-methoxybenzylamino)-2-phenylpiperidine (McLean *et al.*, 1993) (100 nM), used in some guinea-pig Mhb experiments, was synthesized in the Medicinal Chemistry Labs., Parke-Davis.

Results Table 1 shows the results for a series of neurokinin agonists on the firing rate of spontaneously firing guinea-pig Mhb neurones. All (65/65) guinea-pig Mhb neurones were excited in a concentration-dependent fashion by senktide (Figure 1) but none of those tested were affected by the presence of agonists for the NK₁(0/21) or NK₂(0/8) receptor. Further to this the response to senktide was not altered in the presence of CP-99,994 (100 nM) to eliminate any possible interference from NK₁ receptors. A mean EC₅₀ value of 24.5 nM (range 5.0 to 42.0 nM, *n* = 9) was obtained for senktide at NK₃ sites in the guinea-pig Mhb. We compared the relative potencies of senktide and NKB on the same neurones (3 neurones from 3 slices). The mean EC₅₀ values revealed like-potencies for the two agonists (Table 1A). A proportion (approximately 50%) of rat Mhb neurones was excited either by the NK₁ agonist or NK₃ agonist alone (Table 1B). EC₅₀ values of 45.4 nM (range 24.0 to 60.0 nM, *n* = 5) and 76.0 nM (range 16.0 to 220.0 nM, *n* = 5) were obtained for senktide and Sar⁹Met(O₂)¹¹SP respectively in the rat Mhb. Three of 21 neurones in the rat Mhb were excited by both NK₁ and NK₃ agonists.

In contrast to the high incidence of NK₃-receptive neurones in the guinea-pig Mhb, studies of the same region in the gerbil failed to produce any indication of the presence of NK-sensitive neurones in the Mhb. The spontaneous firing

¹ Author for correspondence.

Table 1 Summary of data for NK agonist action on medial habenula neurones of guinea-pig, rat and gerbil

A Guinea-pig					
No of slices	No of neurones	No of neurones responding to tachykinin agonists			Senktide
		Sar ⁹ Met(O ₂) ¹¹ SP	NKB	β-Ala ⁸ NKA(4–10)	
31	65	0/21	5/5	0/8	65/65
EC ₅₀ for senktide = 24.5 nM (5.0–42.0, n = 9)					
EC ₅₀ for senktide cf. NKB (n = 3) = 27.0 nM (4.4–66.0) cf. 39.1 nM (2.7–87.0)					
B Rat					
No of slices	No of neurones	No of neurones responding to tachykinin agonists			Senktide
		Sar ⁹ Met(O ₂) ¹¹ SP		β-Ala ⁸ NKA(4–10)	
18	21	11/21		0/21	9/21
EC ₅₀ for senktide = 45.4 nM (24–60, n = 5)					
EC ₅₀ for Sar ⁹ Met(O ₂) ¹¹ SP = 76.0 nM (16–220, n = 5)					
C Gerbil					
No of slices	No of neurones	No of neurones responding to tachykinin agonists			Senktide
		Sar ⁹ Met(O ₂) ¹¹ SP	SpoMe	β-Ala ⁸ NKA(4–10)	
4	13	0/13	0/13	0/13	0/13
EC ₅₀ values are given at the end of each data set as the mean of n values followed by the range.

Figure 1 Concentration-dependent excitations of a spontaneously firing guinea-pig medial habenula neurone by the two neurokinin (NK) agonists, senktide and NKB: (a) shows a ratemeter histogram of the experiment with the firing rate (Hz) plotted against time of the experiment (seconds). NK agonists were applied for 1 min at the nanomolar concentrations shown ((●) senktide; (○) NKB). (b) Shows the resulting concentration-response curves. The two compounds were approximately equipotent with EC₅₀ values of 4.4 nM (senktide, ●) and 2.7 nM (NKB, ○).

of 13 neurones (4 slices) was unaffected by NK₁, NK₂ or NK₃ agonists, suggesting, few if any, NK-sensitive neurones are present in the gerbil Mhb (Table 1C).

Discussion This study reveals that differences exist in the proportion and type of NK response on neurones within the same brain region in different species. While we were able to confirm that the rat Mhb has both NK₁ and NK₃-receptive neurones, we have also demonstrated for the first time the apparent lack of any functional NK responses in the gerbil Mhb. This is of interest given the recent finding that i.c.v. injection of NK₁ and NK₃ agonists caused hind-limb thumping in the same species (Graham *et al.*, 1993). We also report here for the first time the overwhelming predominance of neurones which are excited by an NK₃ agonist in the guinea-pig Mhb.

The data presented show that the guinea-pig Mhb is an excellent preparation for functional NK₃ receptor studies. The absence of a selective NK₃ antagonist meant that the only test for the presence of NK₃ sites lay in the use of an NK₃-selective agonist combined with an NK₁ antagonist. Under these conditions every guinea-pig Mhb neurone was excited in a concentration-dependent manner by senktide, indicative of a high density of NK₃-sensitive neurones in the guinea-pig Mhb.

The finding that senktide and NKB were approximately equipotent was also important, since this echoes the rank affinities in radioligand binding studies of the two agonists at the cloned human brain NK₃ receptor (Buell *et al.*, 1993). In other rodent brain slice preparations where comparisons of the potencies of NK agonists have been made NKB was considerably less potent than senktide (Seabrook *et al.*, 1992; Norris *et al.*, 1993).

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