Hemokinin 1 is a full agonist at the substance P receptor

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The substance P homolog, hemokinin 1 (HK-1), was identified by differential display as a transcript that is highly expressed in early B cells[1]. It is a predicted 11-aa peptide, generated by proteolytic processing of a hypothetical 128–aa precursor, preprotachykinin C. On the basis of homology with substance P, we hypothesized that HK-1 would interact with the substance P–selective receptor, the neurokinin 1 (NK-1) receptor. Competition binding experiments with 293T cells transfected with the NK-1 receptor showed HK-1 binding with an affinity identical to that of substance P (**Fig. 1**). We also showed HK-1–mediated calcium flux in NK-1 receptor–expressing cells that was equipotent to substance P and cross-desensitized with substance P. Thus, HK-1 appears to interact in a manner similar, if not identical, to substance P at the NK-1 receptor.

In the initial report, HK-1 was shown to have proliferative and anti-apoptotic actions on $B220^+$ cells *in vitro*[1]. Effective concentrations were in the high micromolar range and, because similar activity could not be demonstrated for substance P, the authors postulated the presence of a distinct receptor that is selective for HK-1. However, in nanomolar concentrations, substance P can support hematopoiesis of highly purified human peripheral blood mononuclear cells in the absence of exogenous growth factors[2]. We hypothesized that HK-1 might be more resistant to proteolytic inactivation based on the substitution of tyrosine for phenylalanine at position 8. As a result, HK-1 would be predicted to show biological activity at lower concentrations. As inhibition of neprilysin is critical to maintaining substance P activity[3,4], we compared the degradation rates of substance P and HK-1 by purified neprilysin. Our data show that HK-1 is degraded by this enzyme with kinetics approximately half that of substance P, supporting the stabilizing effect of the amino acid substitution and increased apparent biological activity of HK-1.

The report by Zhang *et al.* also indicated a 90% reduction reduction in pre-B cells in bone marrow and a ~70% reduction in immature B cells in spleens of mice treated with a NK-1R antagonist[1]. We generated NK-$1R^{-/-}$ mice on a BALB/c background[5] and found that the deficiency in NK-1 receptor was also associated with reductions in these cell populations, but only by ~25%. The mouse strain used was the F_1 cross of C57BL/6 with DBA2, which potentially explains the difference in magnitude of the changes in cell populations[1].

In conclusion, we have shown that HK-1 is a full agonist at the NK-1 receptor. We suggest that the earlier report, which postulated a fourth tachykinin receptor based on differences in pre-B cell survival between HK-1 and substance P, was affected by the presence of neprilysin in the system[3,4]. Further, we corroborate a role for tachykinins in regulation of the pre-B cell compartment and extend the observation to show a reliance on the NK-1 receptor. As the NK-1 receptor–deficient mice are not lymphopenic[5], the subtle effect is not due to a loss of precursor B cells. We hypothesize that the absence of NK-1 receptor might somehow accelerate the maturation process and thus limit the size of the pre-B cell pool.

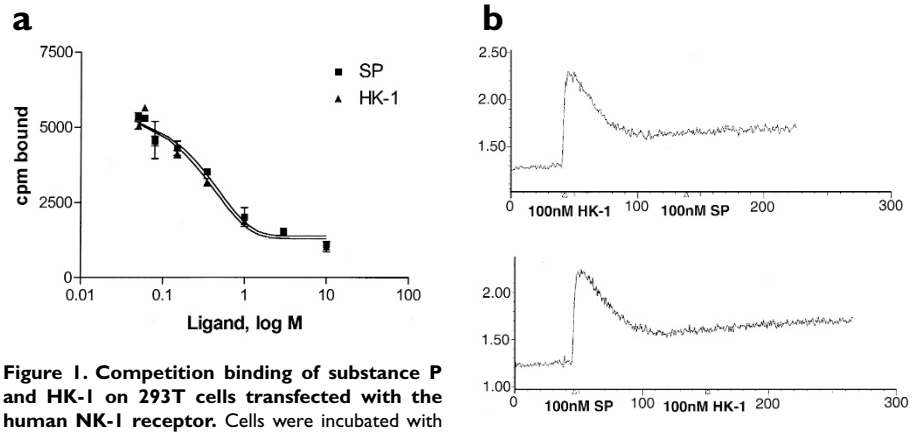

Figure 1. Competition binding of substance P and HK-1 on 293T cells transfected with the human NK-1 receptor. Cells were incubated with 0.05 nM ^{125}I-Bolton Hunter–labeled substance P and unlabeled sunstance P (■) or HK-1 (▲). After 45 min at 22 °C, samples were filtered over glass fiber filters and bound ligand counted. Datapoints were determined in duplicate, and analysis revealed a K_d of 0.16±0.09 nM for substance P and 0.11±0.06 nM for HK-1. Data are representative of four independent experiments. **(b) Representative tracings of the response of Fura–2–loaded 293T cells transfected with the NK-1 receptor to 100 nM HK-1 or substance P.** Cells loaded with Fura-2 were suspended at 2×10^6/ml and placed into the 37 °C cuvette. Substance P or HK-1 was added at the arrows, as indicated. Data are representative of three independent experiments.

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