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Macrophage stimulating protein is a target-derived neurotrophic factor for developing sensory and sympathetic neurons

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SUMMARY

Macrophage stimulating protein (MSP) is a pleiotropic growth factor that signals via the Ron receptor tyrosine kinase. We report that Ron mRNA is expressed by NGF-dependent sensory and sympathetic neurons and that these neurons survive and grow with MSP at different stages of development. Whereas NGF-dependent sensory neurons become increasingly responsive to MSP with age, sympathetic neurons exhibit an early response to MSP that is lost by birth. MSP mRNA expression increases with age in sensory neuron targets and decreases in sympathetic targets. After the phase of naturally occurring neuronal death, significant numbers of NGF-dependent sensory neurons, but not sensory neurons, dependent on other neurotrophins, are lost in mice lacking a functional Ron receptor. These results show that MSP is a target-derived neurotrophic factor for subsets of sensory and sympathetic neurons at different times during their development.

Key words: Mouse, Neuron, MSP,

INTRODUCTION

Several structurally related families of secreted proteins promote and regulate neuronal survival in the developing vertebrate nervous system (Davies, 1994; Ibanez, 1998; Lewin and Barde, 1996). These include the neurotrophins (NGF, BDNF, NT3 and NT4), the GDNF family (GDNF, neurturin, persephin and artemin) and the neurotrophic cytokines (CNTF, LIF, CT1, IL6 and oncostatin M). In addition, hepatocyte growth factor (HGF), a pleiotropic growth factor required for the development of the placenta, liver and skeletal muscle (Birchmeier and Gherardi, 1998; Maina and Klein, 1999), has also recently been shown to enhance the survival of several different types of neurons and promote axonal growth during development (Ebens et al., 1996; Hamanoue et al., 1996; Maina et al., 1997; Maina et al., 1998; Yamamoto et al., 1997; Wong et al., 1997; Yang et al., 1998; Davey et al., 2000; Okura et al., 1999; Novak et al., 2000). Macrophage stimulating protein (MSP) is a secreted protein that has 45% sequence identity to HGF (Leonard and Skeel, 1978; Yoshimura et al., 1993). It stimulates macrophage motility and chemotaxis (Skeel and Leonard, 1994), megakaryocytopoiesis (Banu et al., 1996), keratinocyte proliferation and migration (Wang et al., 1996), and osteoclast activity (Kurihara et al., 1998), and suppresses the proliferation of myeloid progenitor cells (Broxmeyer et al., 1996).

MSP exerts its actions on responsive cells by binding to the receptor tyrosine kinase Ron (Mst1r – Mouse Genome Informatics), which is structurally related to the HGF receptor tyrosine kinase Met (Bottaro et al., 1991; Naldini et al., 1991; Gaudino et al., 1994; Wang et al., 1994). To investigate whether MSP is a neurotrophic factor for developing neurons, we have investigated its actions on sensory and sympathetic neurons cultured at stages throughout their development, and have studied the expression of RON mRNA and MSP mRNA in these neurons and their targets over the same period. We focused on four neuronal populations, the neurotrophic factor requirements of which are very well characterized in the developing mouse. The sensory neurons of the trigeminal and dorsal root ganglia (DRG) are mostly dependent on a supply of NGF from their peripheral targets during the phase of naturally occurring neuronal death (Buchman and Davies, 1993; Memberg and Hall, 1996; Piñón et al., 1996). The sympathetic neurons of the superior cervical ganglion (SCG) are dependent on the supply of both NGF and NT3 during this phase of development (Francis et al., 1999; Wyatt et al., 1997). The sensory neurons of the nodose ganglion depend on BDNF, NT3 or NT4 for survival (ElShamy and Ernfors, 1997) and contain very few NGF-dependent neurons (Forgie et al., 2000). We report that MSP has potent and distinctive, age-related effects on the survival and growth of NGF-dependent neurons, and show that MSP mRNA is expressed in tissues innervated by MSP-dependent neurons.

MATERIALS AND METHODS

Neuron cultures

Dissociated cultures of lumbar DRG, trigeminal ganglia and SCG

neurons were established from CD1 mouse embryos. Dissected ganglia were trypsinized and dissociated by trituration (Davies et al., 1993). The neurons were grown in defined, serum-free medium on a poly-ornithine/laminin substratum in 35 mm diameter tissue culture petri dishes or the 11 mm diameter wells of Greiner four-well dishes. MSP (R & D Systems) and NGF (gift of Gene Burton, Genentech) were added shortly after plating.

To estimate of the number of neurons surviving under different experimental conditions, the number of attached neurons within a 12×12 mm grid in the center of each petri dish was counted 6 hours after plating and was counted again at 48 hours. The number of neurons at 48 hours is expressed as a percentage of the 6 hour count.

To quantify the number of new neurons generated in E14 SCG cultures, an initial cohort that comprised all of the neurons within a 12×12 mm grid was identified 6 hours after plating. The survival of each of these neurons was monitored at 6 hourly intervals and the number of new neurons generated in the same grid was monitored at each time point. The total number of new neurons generated over time in these cultures is expressed as a percentage of the size of the initial cohort. BrdU incorporation into the nuclei of proliferating neuroblasts in E14 SCG cultures was determined immunocytochemically as described previously (Wyatt et al., 1999).

Neurite length was quantified for cultured embryonic neurons by making drawings of neurons with the aid of a drawing tube from which measurements were subsequently made with the aid of a computer-linked digitizing tablet (Davies, 1989). More than 150 neurons were drawn and analyzed for each experimental condition. For the extensive and highly branched neurite arbors of P1 DRG neurons, we used an interactive stereological approach in which the extent of neurite growth was calculated from the frequency at which neurites intersected a series of randomly oriented, computer-generated lines (Kinetics Imaging).

Measurement of MSP and Ron mRNA levels

A quantitative reverse transcription-polymerase chain reaction (RT-PCR) technique (Wyatt and Davies, 1993) was used to measure the levels of MSP and Ron mRNAs in RNA from dissected tissues or purified neurons. The levels of mRNA for the housekeeping protein GAPDH were also determined by quantitative RT-PCR, allowing MSP and Ron mRNA expression to be calculated relative to GAPDH mRNA. The RT- PCR reactions were calibrated by the inclusion of known amounts of cRNA competitor templates for each of the mRNAs in the reverse transcription reaction.

The forward primer for assaying MSP cDNA was 5′-ACC CAG CAC TCG CTG CAC ACA C-3′ and the reverse primer was 5′-TCT GGA AGA GAT GGC ACA GAC TCG-3′. These hybridize 60 bp apart in mouse MSP cDNA and 63 bp apart in the MSP competitor cDNA. The forward primer for amplifying Ron was 5′-ACT GTC ATT TTG CAC CTA AAC GC-3′ and the reverse primer was 5′-AGT GGG CTG CCT GAA GCA CTG-3′. These hybridize 80 bp apart in mouse RON cDNA and 82 bp apart in the RON competitor cDNA. The details of the GAPDH RT-PCR assay and amplification conditions are described elsewhere (Wyatt et al., 1997).

MSP cDNA was amplified by 38 to 40 cycles at 91°C for 60 seconds, 64°C for 45 seconds and 72°C for 60 seconds. Ron cDNA was amplified by 37 cycles of 91°C for 60 seconds, 56°C for 45 seconds and 72°C for 60 seconds. The RT-PCR products of the native MSP, Ron and GAPDH mRNAs, and those of the cRNA competitor species were separated on 8% non-denaturing polyacrylamide gels. These gels were subsequently stained with SyberGold (Cambridge Biosciences) and the intensity of the RT-PCR products were determined using a gel documentation system (Biogene) with Phoretix software.

Quantification of the number of neurons in trigeminal and nodose ganglia

The heads of newborn and P6 pups in litters resulting from matings of *Ron*$^{+/-}$ mice (Correll et al., 1997) were fixed in neutral buffered formalin, decalcified, paraffin wax embedded and serially sectioned at 8 μm through the trigeminal and nodose ganglia. The sections were then stained with Cresyl Fast Violet and neuronal number was quantified using a digital stereology system that employs a combination of the optical dissector and volume fraction/Cavalieri methods (Kinetic Imaging).

RESULTS

MSP is an increasingly potent neurotrophic factor for NGF-dependent sensory neurons during development

We began investigating the neurotrophic actions of MSP by studying its effect on DRG neurons in low density dissociated cultures established from mouse embryos over a range of developmental stages. These experiments revealed that MSP became increasingly effective in promoting survival with age. In cultures established from E12 embryos, MSP supported less than 10% of the neurons, while by postnatal day 1 (P1), over 60% of the neurons survived with MSP (Fig. 1). At both ages, NGF promoted the survival of approximately 80% of the neurons, but no additional neurons survived with MSP plus NGF, indicating that the MSP-responsive neurons comprise an increasing subset of the NGF-responsive neurons in these ganglia and that these neurons start responding to MSP after they have become NGF responsive. Because HGF enhances the survival of sensory neurons grown with NGF (Maina et al., 1997), we investigated the combined effect of MSP and HGF on sensory neuron survival. At no age were there significantly more neurons surviving with MSP plus HGF compared with MSP alone (data not shown).

To further characterize the survival-enhancing effects of MSP on developing DRG neurons, we studied the dose-response relationship between these neurons and MSP at

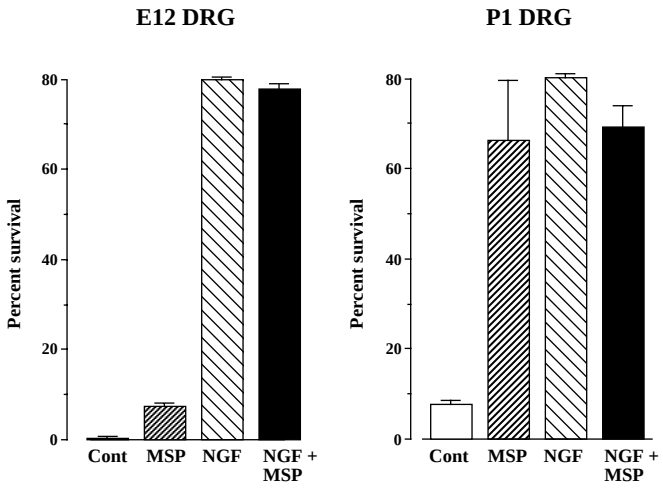

Fig. 1. Comparison of the effects of MSP and NGF on the survival of DRG neurons during development. Bar charts of the percent survival of E12 and P1 DRG neurons after 48 hours incubation in defined medium alone (Cont) or medium supplemented with 10 ng/ml MSP, 10 ng/ml NGF or MSP plus NGF. The means and standard errors for data obtained from three petri dishes for each experimental condition are shown.

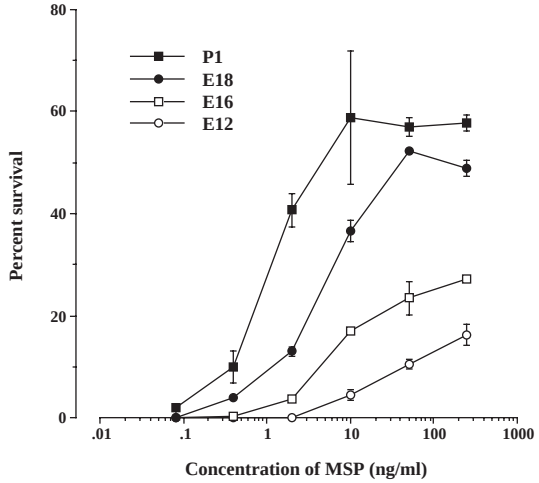

Fig. 2. Developmental changes in the dose responses of DRG neurons to MSP. Graph of the percent survival of E12, E16, E18 and P1 DRG neurons after 48 hours incubation with a range of concentrations of MSP. The means and standard errors for data obtained from three petri dishes for each experimental condition are shown.

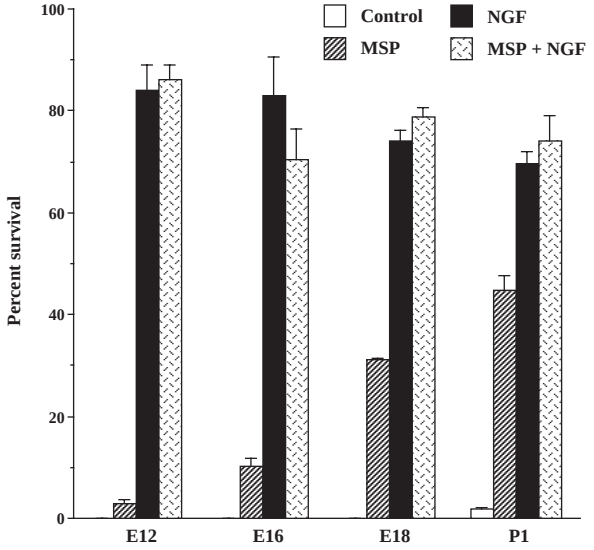

Fig. 3. Comparison of the effects of MSP and NGF on the survival of trigeminal ganglion neurons during development. Percent survival of E12, E16, E18 and P1 trigeminal ganglion neurons after 48 hours incubation in defined medium alone (Control) or medium supplemented with 10 ng/ml MSP, 10 ng/ml NGF or MSP plus NGF. The means and standard errors for data obtained from three petri dishes for each experimental condition are shown.

different developmental stages. These experiments not only showed that MSP promoted the survival of increasing numbers of DRG with age, but that the neurons became increasingly sensitive to MSP with age. The MSP dose-response curve exhibited a marked shift to the left between E12 and P1 (Fig. 2). Interpolation of the data revealed an approximate 25-fold decrease in the EC_{50} over this period of development.

We also studied the effect of MSP on trigeminal ganglion neurons, a population of cutaneous sensory neurons that also contains a large proportion of NGF-dependent neurons. Fig. 3 shows that the effectiveness with which MSP promoted the survival of these neurons also increased with age: from less than 5% survival in E12 cultures to 65% of the number of neurons surviving with NGF by P1 (Fig. 3). During this period of development, NGF promoted the survival of the majority of the neurons. As in DRG cultures, the number of neurons that survived with MSP plus NGF was not significantly greater than the number surviving with NGF alone (P>0.05, t-test), indicating that MSP promotes the survival of an increasing proportion of NGF-responsive neurons in the trigeminal ganglion with age. In contrast to DRG and trigeminal neurons, the survival of nodose ganglion neurons in the presence of 10 or 50 ng/ml of MSP was not significantly greater than controls at E14, E16 or P1 (data not shown).

MSP is an early survival factor for a subset of sympathetic neurons

To investigate if MSP influences sympathetic neuron survival, we established low density SCG cultures at stages from E14 (when the SCG still contains many proliferating neuroblasts and immature neurons that survive for several days without added neurotrophic factors) to P1 (when most of the neurons have become dependent on NGF for survival). In marked contrast to sensory neurons, sympathetic neurons showed a survival response to MSP that decreased with age. In E14 cultures, there were 20% more neurons surviving with MSP than in control cultures. By E17, there were fewer than 10% more neurons with MSP, and by P1 there were almost no neurons with MSP. There was no significant difference between the number of neurons with MSP plus NGF compared with the number with NGF alone (P>0.05, t-test). Dose-response analysis showed that the maximally effective concentration of MSP for E14 SCG survival was 10 ng/ml (Fig. 4), the same concentration that is maximally effective for P1 DRG neuron survival (Fig. 2).

Because the E14 SCG contains many proliferating neuroblasts that differentiate into neurons in culture, we carried out additional experiments to determine if the increased number of neurons in MSP supplemented cultures was due to increased neurogenesis. We estimated the number of new neurons generated over time in culture by identifying all of the neuroblasts and neurons in a grid in the center of culture dishes 6 hours after plating. By following the fate of each of these neurons at six hourly intervals, we were able to recognize and count all new neurons that were generated in this grid over time. These experiments showed that there was no significant difference in the total number of new neurons that were generated between 6 and 48 hours (expressed as a percentage of the initial 6 hour cohort) in control cultures and cultures supplemented with MSP (12.1±0.4% and 13.1±1.2%, respectively, n=3 experiments, P>0.05). These cohort experiments, however, revealed that MSP enhanced the survival of post-mitotic neurons. Immunocytochemical measurement of BrdU incorporation in these cultures also revealed no significant difference between the numbers of BrdU-positive cells in control and MSP-supplemented cultures (20.9±1.4% and 22.7±1.3%, respectively, after 18 hours incubation with BrdU, n=3 experiments, P>0.05). These results show that MSP neither promotes sympathetic neuroblast proliferation nor enhances sympathetic neuron

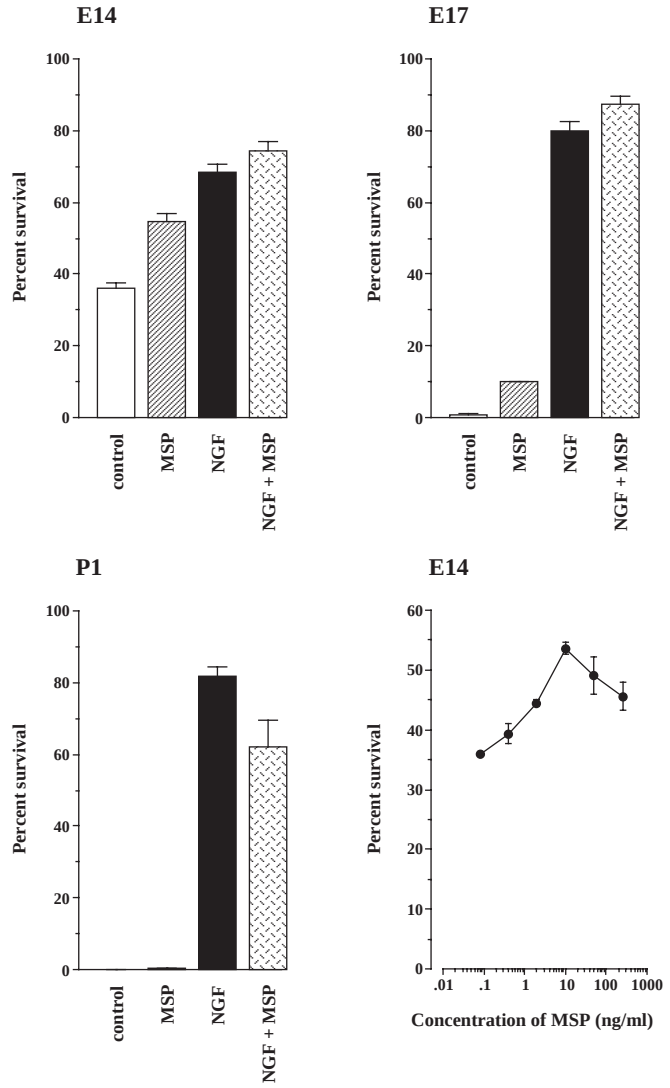

Fig. 4. Comparison of the effects of MSP and NGF on the survival of SCG neurons during development. Percent survival of E14, E17 and P1 SCG neurons after 48 hours incubation in defined medium alone (Control) or medium supplemented with 10 ng/ml MSP, 10 ng/ml NGF or MSP plus NGF. Graph of the percent survival of E14 SCG neurons after 48 hours incubation with a range of concentrations of MSP. The means and standard errors for data obtained from three petri dishes for each experimental condition are shown.

generation, and demonstrate that the reason for the increased number of neurons in early SCG cultures is due to a survival effect of MSP on newly differentiated, post-mitotic sympathetic neurons.

MSP enhances neurite growth from sensory and sympathetic neurons

To investigate the effects of MSP on neurite growth it was essential to compare the same subset of neurons with and without MSP. Because MSP-responsive neurons comprise a subset of NGF-responsive neurons, we grew neurons with NGF in the presence or absence of MSP and estimated the total length and number of branch points in the neurite arbors that grew from the neurons. Fig. 5 shows that in P1 DRG cultures

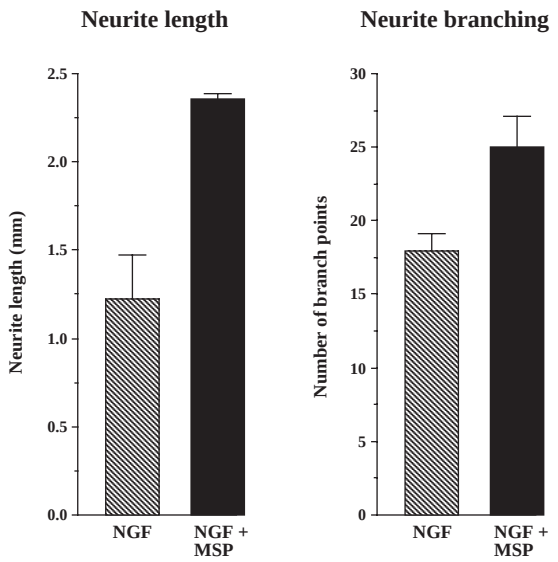

Fig. 5. The total length and number of branch points in the neurite arbors growing from P1 DRG neurons after 24 hours incubation with NGF alone or NGF plus MSP (both at 10 ng/ml). The means and standard errors of the data from three separate experiments for length measurements and means and standard errors of branch point counts from 110 neurons in each condition are shown.

after just 24 hours incubation, MSP promoted a 38% increase in the average length of neurite arbors and a 39% increase in branch point number. Although there was no significant effect of MSP on the growth of P1 SCG neuron arbors (data not shown), in E14 SCG cultures, MSP promoted a small, statistically significant increase in neurite length (3.65±0.16 mm versus 4.21±0.16 mm with NGF and NGF+MSP, respectively, $P<0.01$) and branching (5.20±0.32 versus 6.03±0.35 branches with NGF and NGF+MSP, respectively, $P<0.05$) after 48 hours incubation. These results show that MSP enhances the growth and branching of sensory and sympathetic neurites during the stages of development when these neurons survive with MSP.

MSP mRNA and Ron mRNA in neurons and their targets

To ascertain the source of MSP for developing sensory neurons in vivo, we used competitive RT-PCR to measure the level of MSP mRNA in the trigeminal ganglion and a well defined part of its cutaneous territory at stages throughout its innervation. The earliest trigeminal axons reach the maxillary process by E11, and over the next 5 days this structure and the mystacial whisker pad that develops from it becomes the most densely innervated cutaneous tissue in the mouse embryo (Davies and Lumsden, 1984). MSP mRNA was detected in the maxillary territory as early as E12 and its level increased markedly with age as trigeminal neurons become responsive to MSP and their axons reach and ramify within this tissue (Fig. 6). By contrast, the level of MSP mRNA in the iris, a structure that receives a dense sympathetic innervation from the SCG, was initially high during the earliest stages of its innervation, but subsequently fell markedly later in embryonic development. Compared with the high levels of MSP mRNA in the target fields of sensory and sympathetic neurons at the stages of development when

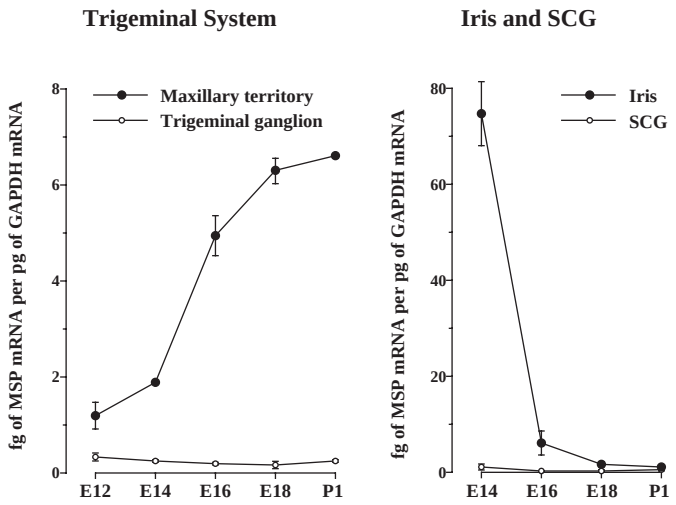

Fig. 6. Developmental changes in the expression of MSP mRNA in the trigeminal ganglion and SCG, and in the maxillary target field and iris at different developmental stages. The level of MSP mRNA relative to GAPDH mRNA in the maxillary process at E12 and the mystacial whisker pad that develops from it at E14, E16, E18 and P1. The level of MSP mRNA in whole trigeminal ganglia is also shown at each of these ages. The mean and standard error of at least three data points at each age are shown.

these neurons are most sensitive to MSP, the levels of MSP mRNA in the trigeminal ganglia and SCG were negligible. Moreover, in contrast to the marked developmental changes in MSP mRNA in target tissues, there were no developmental changes in MSP mRNA expression in these ganglia. These results suggest MSP is a target-derived neurotrophic factor for sensory and sympathetic neurons at different stages of target field innervation.

RT-PCR analysis revealed that developing trigeminal ganglia and SCG both express Ron mRNA. Because at E16 and later there is a sufficient size difference between neurons and non-neuronal cells in the trigeminal ganglion to permit neuronal purification by differential sedimentation (Davies, 1986), we were able to show that neuronal expression of Ron mRNA increases from E16 to P1 as neurons become more sensitive to MSP (53.2±9.7 and 122.3±18.7 fg Ron mRNA per pg GAPDH mRNA at E16 and P1, respectively, n=3). Furthermore, we also compared the relative levels of Ron mRNA in whole DRG, SCG and nodose ganglia at P1 by semi-quantitative RT/PCR. Compared with the level of Ron mRNA in MSP-responsive DRG, the levels of Ron mRNA in the SCG and nodose ganglia were much lower (relative to GAPDH mRNA levels, the levels of Ron mRNA in the SCG and nodose ganglia were a half and one fifth the level in the DRG, respectively). Thus, ganglia that contain large numbers of neurons that survive in response to MSP contain relatively higher levels of Ron mRNA than ganglia that contain few MSP-responsive neurons.

Reduction of sensory neurons in mice with defective MSP signaling

To ascertain the physiological relevance of our in vitro findings, we studied the in vivo consequences of defective MSP signaling via its specific receptor tyrosine kinase Ron. Because complete elimination of Ron leads to early embryonic death (Muraoka et al., 1999), we studied mice with a targeted deletion of the ligand-binding domain (Correll et al., 1997). Although these mice do not express the Ron receptor on the cell surface, they possess a truncated receptor consisting of the transmembrane and tyrosine kinase domains whose expression is driven by an internal promoter (Correll et al., 1997; Persons et al., 1999). Mice homozygous for this mutant survive into adulthood with deregulated inflammatory responses caused by impaired MSP signaling in macrophages (Correll et al., 1997; Morrison and Correll, 2002; Lutz et al., 2002).

We compared the number of neurons in the trigeminal ganglia $Ron^{-/-}$ and wild-type mice in newborn and P6 neonates (Fig. 7). In newborn mice, there were no fewer neurons in $Ron^{-/-}$ mice than in wild types. However, between P1 and P6 there was a highly significant 25% decrease in the number of neurons in the trigeminal ganglia of $Ron^{-/-}$ mice (P<0.001, t-test), whereas over the same period of development there was no significant change in the number of neurons in $Ron^{+/+}$ mice (<1% decrease, P>0.05, t-test), and at P6 there were significantly fewer neurons in the trigeminal ganglia of $Ron^{-/-}$ mice compared with $Ron^{+/+}$ mice (P< 0.01, t-test). Although there appear to be more neurons in the trigeminal ganglia of $Ron^{-/-}$ mice compared with $Ron^{+/+}$ mice, this difference was not statistically significant (P>0.05). Taken together, these data suggest that neurons are lost in the trigeminal ganglia of $Ron^{-/-}$ mice in the postnatal period, implying that MPS/Ron signaling is important for sustaining the survival of a subset of these neurons in vivo after the period of naturally occurring neuronal death that is over by birth (Davies and Lumsden, 1984). To ascertain whether the loss of trigeminal neurons in the postnatal period in $Ron^{-/-}$ mice is specific for MSP-responsive neurons or is a consequence of a generalized effect of defective Ron signaling on neuronal survival, we compared the number of neurons in the nodose ganglion of P6 wild type and $Ron^{-/-}$ mice. In accordance with the lack of MSP on the in vitro survival of embryonic and postnatal nodose neurons, there were no fewer neurons in the nodose ganglia of $Ron^{-/-}$ mice than wild-type mice at P6 (Fig. 7).

DISCUSSION

We have shown that MSP is a novel neurotrophic factor for sensory and sympathetic neurons that acts during different stages of their development. Whereas MSP promotes the survival of an increasing proportion of DRG and trigeminal sensory neurons with age, it promotes the survival of a subset of sympathetic neurons shortly after they differentiate from neuroblasts but not at later embryonic stages. MSP starts to promote the survival of DRG and trigeminal neurons shortly after most of these neurons have become dependent on NGF for survival at E12. The proportion of MSP-responsive neurons in these ganglia increases throughout the second half of embryonic development until, by birth, the majority of NGF-responsive neurons also respond to MSP. Throughout this period of development, which encompasses the phase of naturally occurring neuronal death (Davies and Lumsden, 1984; Piñón et al., 1996), the sensitivity of the neurons to MSP increases by over two orders of magnitude. Our finding that there is no additional neuronal survival in cultures containing

MSP plus NGF compared with NGF alone suggests that MSP is an increasingly potent neurotrophic factor for NGF-dependent sensory neurons during the period of naturally occurring neuronal death. Accordingly, the BDNF- and NT4-dependent neurons of the nodose ganglion are not supported by MSP in culture. Our demonstration that there are significantly fewer neurons in the trigeminal ganglia than in the nodose ganglia of postnatal mice that lack a functional MSP receptor shows that MSP is a physiologically relevant survival factor for a subset of NGF-dependent sensory neurons in vivo. Because the period of naturally occurring neuronal death is over by birth in the trigeminal ganglion (Davies and Lumsden, 1984), our finding that the loss of neurons in the trigeminal ganglia of *Ron*$^{-/-}$ mice occurs between birth and P6 suggests that MSP is not directly involved in regulating the final number of neurons in the ganglion but is important for sustaining the number that remains after target selection has taken place.

The level of MSP mRNA increases in the maxillary territory of the trigeminal ganglion over the same period of development as trigeminal neurons acquire responsiveness to MSP, reaching a plateau by P1 when the neurons have become maximally responsive to this factor. Because the level of MSP mRNA is negligible in the trigeminal ganglion, these results suggest that MSP is, like NGF, a target-derived neurotrophic factor for a subset of cutaneous sensory neurons. The level of NGF mRNA also increases in the maxillary territory during its innervation by the trigeminal ganglion, but reaches a plateau at E15 (Davies et al., 1987), several days before the level of MSP mRNA reaches its plateau. This sequential rise in the expression of NGF and MSP mRNAs in the trigeminal territory accords with the sequential development of NGF and MSP responsiveness with age.

Although NGF is capable of promoting the survival of the majority of trigeminal neurons in culture throughout the period of naturally occurring neuronal death, the NGF dose-response curve shifts by over an order of magnitude to higher NGF concentrations during this period of development, indicating that the neurons become much less sensitive to NGF with age (Buchman and Davies, 1993). This observation, together with our finding that sensory neurons become much more sensitive to MSP over the same period of development, suggests a developmental switch in responsiveness from NGF to MSP during development. Indeed, the finding that the great majority of trigeminal neurons die by E15 in mice that lack functional TrkA receptors (Piñón et al., 1999) suggests that NGF/TrkA signaling is particularly important for sustaining neuronal survival during the early stages of naturally occurring neuronal death.

The concept of neurotrophic factor switching originated from work on the early mouse trigeminal system, and has since been observed in several other populations of neurons. Most E10 and E11 trigeminal neurons survive in culture with BDNF and very few survive with NGF, whereas by E12 most neurons survive with NGF and very few survive with BDNF (Buchman and Davies, 1993; Paul and Davies, 1995). Although this switch in responsiveness is due in part to the sequential generation of BDNF-responsive and NGF-responsive neurons (Enokido et al., 1999; Huang et al., 1999), many of the neurons that initially survive with BDNF subsequently switch to become NGF-responsive (Enokido et al., 1999). Thus, in the trigeminal ganglion, there appears to be a switch from BDNF to NGF dependence early in development followed by a later shift in responsiveness from NGF to MSP during the period of naturally occurring neuronal death.

In contrast to developing sensory neurons, sympathetic neurons exhibit a transient survival response to MSP shortly after they differentiate from their precursor cells, but not at later stages during the phase of naturally occurring neuronal death. Accordingly, MSP mRNA is expressed at high levels in the iris, a densely innervated sympathetic target, at this early stage in development, and its expression decreases markedly with age. These findings raise the possibility that MSP is required to sustain the survival of a subset of sympathetic neurons during the early stages of target field innervation.

MSP is structurally related to HGF, a pleiotropic factor that has a well established neurotrophic factor role in the developing nervous system (Ebens et al., 1996; Hamanoue et al., 1996; Maina et al., 1997; Maina et al., 1998; Yamamoto et al., 1997; Wong et al., 1997; Yang et al., 1998; Davey et al., 2000; Okura et al., 1999; Novak et al., 2000). However, the neurotrophic actions of HGF differ in an important respect from those of MSP. Whereas HGF generally only enhances neuronal survival in the presence of other neurotrophic factors, MSP increases the survival of sensory and sympathetic neurons

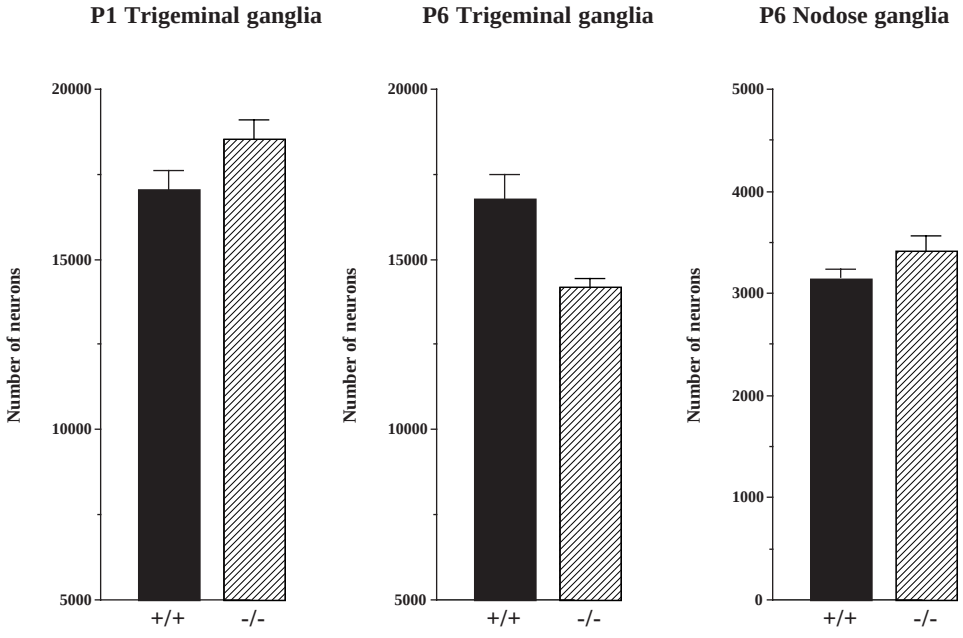

Fig. 7. Total numbers of neurons in P1 and P6 trigeminal ganglia and P6 nodose ganglia of *Ron*$^{+/+}$ and *Ron*$^{-/-}$ mice. The mean and standard errors are shown (n=4 wild type at both ages; n=4 *Ron*$^{-/-}$ at both ages).

in the absence of other neurotrophic factors. Moreover, for sensory neurons at least, MSP is a far more effective and potent neurotrophic factor than HGF. MSP has also recently been shown to promote the survival of embryonic hypoglossal motoneurons (Schmidt et al., 2002) and sustain cholinergic acetyltransferase expression in these neurons after axotomy in the adult (Stella et al., 2001).

In summary, we have shown that MSP is a target-derived neurotrophic factor that promotes the survival and growth of sensory and sympathetic neurons at defined stages in their development. Its neurotrophic effects differ from those of the structurally related factor HGF in having direct survival-promoting effects independently of the presence of other factors.

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