Journal of Physiology (1993), **471**, *pp.* 209–221
With 5 figures
Printed in Great Britain

HYPOTHALAMIC MODULATION OF SPLENIC NATURAL KILLER CELL ACTIVITY IN RATS

BY TOSHIHIKO KATAFUCHI, TOMOYASU ICHIJO, SACHIKO TAKE AND TETSURO HORI

From the Department of Physiology, Faculty of Medicine, Kyushu University 60, Fukuoka 812, Japan

(*Received 11 January 1993*)

SUMMARY

1. The cytotoxic activity of splenic natural killer cells measured by a standard chromium release assay in urethane and α-chloralose-anaesthetized rats was significantly suppressed 20 min after bilateral ablation of the medial part of the preoptic hypothalamus (MPO). The suppression was completely blocked by prior splenic denervation. The splenic natural killer cell activity of MPO sham-lesioned rats or thalamus-lesioned rats, both having an intact splenic innervation, were not different from that of a non-treated control group.

2. Electrical stimulation of the bilateral MPO (0·1 ms, 0·1–0·3 mA, 5–100 Hz) suppressed the efferent activity of the splenic nerve in all six rats examined. The reduction of the nerve activity was accompanied by a transient fall in blood pressure. An I.V. injection of phenylephrine (3 μg/0·3 ml) also evoked a suppression of the nerve activity, which was accompanied by transient hypertension, suggesting that the suppressive effect of the MPO stimulation was independent of changes in blood pressure. On the other hand, a bilateral lesion of the MPO resulted in a sustained increase in the electrical activity of the splenic sympathetic nerve filaments which lasted for more than 2 h.

3. Microinjection of monosodium-L-glutamate (0·1 and 0·01 M in 0·1 μl saline) unilaterally into the MPO evoked a transient suppression of the efferent discharge rate of the splenic nerve activity within 1 min, which was also accompanied by a decrease in blood pressure. The injection of saline (0·1 μl) into the MPO had no effect. The microinjection of recombinant human interferon-α (200 and 2000 U in 0·1 μl saline) into the MPO dose dependently increased the splenic nerve activity without any change in blood pressure.

4. In contrast, microinjection of interferon-α into the paraventricular nucleus of the hypothalamus (PVN) had no effect on splenic nerve activity, although an injection of glutamate increased the nerve activity.

5. The present results, taken together with previous reports, suggest that the neuronal networks between the MPO and the splenic sympathetic nerve, which may be activated by centrally administered interferon-α, are important in the suppression of the splenic cellular immunity.

MS 2033

INTRODUCTION

The involvement of the central nervous system in the modulation of immune functions has been demonstrated by a variety of brain lesioning and pharmacological studies. In particular, the destruction of discrete sites in the hypothalamus, limbic structures, brainstem, and the cerebral cortex has been shown to result in either enhancement or suppression of certain immune responses depending on the site and the timing of the lesioning (Roszman, Cross, Brooks & Markesbery, 1985; Jankovic & Spector, 1986; Labeur, Nahmod, Finkielman & Arzt, 1991). The most remarkable and consistent changes in immunological parameters were observed after the lesion of the medial part of the preoptic hypothalamus (MPO), which resulted in a decrease in the blastogenic responsiveness to a mitogen, Concanavalin A (Cross, Brooks, Roszman & Markesbury, 1982). Moreover, the cytotoxic activity of natural killer cells, which is important in immune surveillance, was suppressed in the spleen 4–7 days after lesioning the MPO (Cross, Markesbery, Brooks & Roszman, 1984). Microinjection of β-endorphin into the MPO has also been shown to produce a significant suppression of splenic natural killer cell activity (Hori, Take, Mori, Kaizuka, Shimizu & Katafuchi, 1991*b*). The MPO contains neurones which directly or indirectly receive signals from the immune system. Thermosensitive neurones in the MPO respond to local application of immune cytokines such as interleukin-1β and interferon-α both *in vivo* and *in vitro*, in appropriate ways to explain the cytokine-induced fever (see review by Hori, Nakashima, Take, Kaizuka, Mori & Katafuchi, 1991*a*). These findings suggest an important role for the MPO in bidirectional communication between the brain and the immune system.

We have demonstrated that an I.C.V. injection of recombinant human interferon-α, which increases the splenic sympathetic nerve activity (Katafuchi, Hori & Take, 1991; Katafuchi, Take & Hori, 1993), also results in a suppression of splenic natural killer cell activity 30 min after the injection in rats (Hori *et al.* 1991*b*) and mice (Take, Mori, Katafuchi, Kaizuka & Hori, 1992*a*). The suppression was completely blocked by surgical denervation of the splenic sympathetic nerve. In addition, electrical stimulation of the splenic sympathetic nerve reduced the cytotoxicity of natural killer cells in the spleen (Katafuchi *et al.* 1993). These results suggest that the splenic sympathetic nerve modulates the mechanisms of peripheral cellular immunity. To study further the central mechanisms of the immunosuppression mediated by the splenic sympathetic nerve, we examined: (1) the effects of electrical lesioning of the MPO on the cytotoxic activity of the splenic natural killer cells, (2) responses of the efferent discharges of the splenic nerve to electrical stimulation and lesioning of the MPO and (3) responses to chemical stimulation of the MPO achieved by microinjection of monosodium-L-glutamate or recombinant human interferon-α, which were compared with the effects of these substances injected into the paraventricular hypothalamic nucleus (PVN). Preliminary results of this study have been presented previously (Katafuchi, Take, Ichijo & Hori, 1992).

METHODS

Animals

Male Wistar–King A rats (Laboratory of Animal Experiments, Faculty of Medicine, Kyushu University) weighing 250–300 g were used. They were housed two or three per cage in a room

maintained at a constant temperature (21–23 °C) and relative humidity (60–70%) with lights on from 8.00 to 20.00 h. Standard laboratory chow and tap water were available *ad libitum*.

Measurement of splenic natural killer cell activity following the MPO lesion

Implantation of the electrodes. Under pentobarbitone sodium anaesthesia (50 mg/kg, I.P.), the rats ($n = 12$) were stereotaxically implanted with concentric bipolar stainless steel electrodes (a 26-gauge outer tube containing an inner 0·1 mm diameter wire with tip-separation of 0·5 mm) into the bilateral MPO (A, 0·6 mm posterior to the bregma; L, 1·0 mm from the midline; H, 8·5 mm from the surface of the brain) according to the atlas of Paxinos & Watson (1986). In another four rats, bilateral electrodes were implanted into the thalamus (A, 2·3 mm; L, 1·5 mm; H, 6·5 mm). The electrodes were then cemented to the skull.

Denervation of the splenic nerve. In four of the twelve rats, the denervation of the splenic nerve was performed immediately after the implantation of the electrodes into the MPO. Following laparotomy, the splenic nerve was isolated from the splenic artery, the portal vein and the connective tissues near the bifurcation of the coelaic artery, then the entire bundle of the nerve was cut. Since there are no cholinergic nerve terminals and no afferent innervation in the spleen (Bellinger, Felten, Lorton & Felten, 1989; Nance & Burns, 1989; Felten & Felten, 1991), the nerve fibres innervating the spleen are considered to consist mainly of sympathetic fibres. The remaining eight MPO-implanted rats and four thalamus-implanted rats were also laparotomized and their splenic nerves were isolated, but not denervated (sham-denervation). After surgery, all rats were administered sulphadimethoxide (100 mg/rat, I.P.) and then returned to their home cages.

Measurement of the cytotoxic activity of splenic natural killer cells. One week after surgery, each animal was anaesthetized with an I.P. injection of a mixture of urethane (760 mg/kg) and α-chloralose (65 mg/kg). The animals were divided into four groups (each group, $n = 4$); the MPO-lesioned but sham-denervated group, the MPO-lesioned and splenic-denervated group, the MPO sham-lesioned and sham-denervated group, and the thalamus-lesioned but sham-denervated group. In addition to the twelve rats, four rats that were not operated on at all except for anaesthesia served as the controls. An electrical lesion of the MPO or the thalamus was made by passing a direct current of 1 mA for 15 s.

The spleen was surgically removed 20 min after the lesioning in the MPO- or the thalamus-lesioned rats, or the corresponding period after anaesthesia in both the sham-lesioned rats and the control rats. A single-cell suspension of splenocytes was made by a mechanical dissociation of the spleen. Non-adherent mononuclear cells were collected by passing the cell suspension through a G10 column. After being washed four times in phosphate-buffered saline, the viable cell populations which contained > 95% lymphocytes and < 5% monocytes by Wright's staining, were counted by Trypan Blue dye exclusion. Cells were resuspended (10^7 cells per ml) in complete media RPMI 1640 containing 10% heat-inactivated fetal bovine serum and were used as the effector cells.

The cytotoxic activity of natural killer cells was measured by a standard 4 h chromium release assay. YAC-1 murine lymphoma target cells were labelled for 1 h with ^{51}Cr ($Na_2{}^{51}CrO_4$, Japan Atomic Energy Research Institute) and adjusted to 10^5 cells per ml. YAC-1 cells were then distributed in the wells of ninety-six-well microtitre plates (10^4 cells in 100 μl medium in each well). A suspension of effector cells (in 100 μl medium) was added to each well at an effector:target cell ratio of 100:1. After a 4 h incubation of the effector–target cells mixture at 37 °C in 5% CO_2, the plates were centrifuged at 1000 *g* for 10 min and the supernatant was removed from each well. The ^{51}Cr released from lysed targets was counted in a gamma counter. The percentage of specific lysis was calculated as follows:

$$\text{Per cent cytotoxicity} = \frac{\text{Experimental release–spontaneous release}}{\text{Maximum release–spontaneous release}} \times 100.$$

The spontaneous release resulted from background counts released by ^{51}Cr-labelled target cells alone. The maximum release was determined by incubating target cells with 10% octyl phenoxypolyethoxyethanol. All samples were assayed in quadruplicate. The individual scores were transformed to the percentage of the mean value of the controls (relative natural killer cell activity), and then they were pooled.

Recording of the electrical activity of the splenic sympathetic nerve

The rats used in this experiment were implanted with bilateral electrodes into the MPO, which were used for the electrical stimulation and/or lesion of the MPO.

One week after surgery, each animal was anaesthetized with an I.P. injection of a mixture of

urethane (760 mg/kg) and α-chloralose (65 mg/kg). The left femoral artery and vein were cannulated for the continuous measurement of blood pressure and infusion of drugs, respectively. Rectal temperature was monitored by a thermistor probe and maintained at 36–37 °C by an automatically regulated heating pad.

Following laparotomy, the splenic nerve was isolated and cut near the spleen. The central cut end of the nerve was dissected into fine filaments and placed on a pair of platinum wire electrodes. The nerve filaments and the recording electrodes were immersed in a mixture of liquid paraffin and white Vaseline. The efferent discharges of the nerve filaments were amplified by means of a capacitor-coupled differential preamplifier (DAM-5A, W-P Instruments Inc., New Haven, CT, USA), and were displayed on a storage oscilloscope to be photographed. Multifibre spikes were picked up by a window discriminator, assuring that no electrical noise was picked up, and were integrated with a reset time of 2 or 5 s. The discharge rates and the blood pressure were displayed on a polygraph.

When stable recording of the nerve activities had been established for at least 30 min, the MPO was bilaterally stimulated with rectangular pulses (duration, 0·1 ms; intensity, 0·1–0·3 mA; frequency, 5–100 Hz) for 1 or 2 min and the responses of the splenic nerve filaments were observed. In some cases, phenylephrine, a peripheral vasoconstrictor, was administered I.V. (3 μg in 0·3 ml saline) to investigate the effects of hypertension on the nerve activity. At the final stage of each experiment, the effects of an electrolytic lesion of the bilateral MPO on the nerve activity was observed for more than 1 h. The MPO lesioning was performed by passing a direct current (1 mA, for 15 s) through the stimulation electrodes.

Splenic sympathetic nerve activity following the microinjection of monosodium-L-glutamate and interferon-α

Implantation of the microinjection cannula. Under pentobarbitone sodium anaesthesia (50 mg/kg, I.P.) a 23-gauge stainless steel guide cannula was stereotaxically implanted. The tip of the cannula was placed 1·0 mm above the left MPO (the co-ordinates, same as the stimulating electrode mentioned above) or PVN (A, 1·8 mm posterior to the bregma; L, 0·5 mm; H, 7·5 mm from the brain surface; Paxinos & Watson, 1986) ($n = 3$, respectively). The cannula was fixed to the skull with dental cement. A stainless-steel wire (diameter, 100 μm) of the same length was inserted into the cannula as a stylet.

Infusion of the drugs into the MPO and the PVN. One week after the operation, the splenic sympathetic nerve activity of the anaesthetized rats was recorded as described above and its responses to drugs microinjected into the hypothalamus were studied. After removing a stylet, monosodium-L-glutamate (0·01–0·1 M in 0·1 μl saline), recombinant human interferon-α (200 and 2000 U in 0·1 μl saline, kindly donated by the Japan Roche Pharmac. Co.) or the same amount of saline was administered over a 30 s period during recordings of the nerve activity and blood pressure. The infusion was done through a 29-gauge injection needle which was connected with a 1 μl Hamilton syringe. The tip of the injection needle was inserted 1·00 mm beyond the tip of the guide cannula.

Analysis of data

Responses of the splenic nerve activity to electrical stimulation or lesioning, and the application of drugs were considered to be significant if the mean firing rate for the 1 min period of the most prominent response changed by at least 30% from its basal firing level (mean firing rate during 5 min).

All values for the splenic natural killer activity were given as the means ± S.E.M. Statistical analyses of the data were carried out by repeated measures analysis of variance (ANOVA) followed by Newman–Keuls' multiple-range test for critical differences. A P value of less than 0·05 was considered to be statistically significant.

Histology

After completion of experiments, the animals were microinjected with 0·1 μl of Pontamine Sky Blue acetate. Subsequently, all the animals were perfused transcardially with saline followed by 10% neutral formaldehyde. The brains were removed and stored in 30% sucrose for at least 24 h. Each brain was then cut into 120 μm serial sections on a freezing microtome and stained with Neutral Red. The sites of electrolytic lesion and the distribution of the dye in the brain were verified microscopically.

RESULTS

The effects of the MPO lesion on the cytotoxicity of splenic natural killer cells

In the control animals that had not received any treatment except for anaesthesia, the percentage specific lytic activity of the splenic natural killer cells was $35{\cdot}6 \pm 2{\cdot}5\%$ (mean $\pm$ S.E.M., $n = 4$) at the effector:target cell ratio of 100:1. Values of natural killer cell activity in treated animals were expressed as a percentage of the mean values of the non-treated control rats (the relative natural killer cell activity).

In the control group, the individual values of the relative natural killer cell activity ranged from 87·3–119·8% ($100{\cdot}0 \pm 7{\cdot}1\%$, $n = 4$) of the mean value of the group. There was a significant difference in the relative natural killer cell activity among the different groups ($F(4, 19) = 3{\cdot}90$, $P < 0{\cdot}05$, one-way ANOVA). Individual comparison indicated that the cytotoxicity of the splenic natural killer cells of the rats which received the MPO lesion with sham-denervation of the spleen was significantly reduced to $32{\cdot}6 \pm 16{\cdot}4\%$ ($n = 4$) of the control value ($P < 0{\cdot}05$, Newman–Keuls' test). However, splenic denervation completely abolished the MPO lesion-induced suppression of the natural killer cell activity (the relative natural killer cell activity of the MPO-lesioned and splenic-denervated group, $101{\cdot}8 \pm 18{\cdot}3\%$, $n = 4$). Neither the MPO sham-lesion with sham-denervation group ($102{\cdot}5 \pm 30{\cdot}2\%$, $n = 4$) nor the thalamus lesion with sham-denervation group ($158{\cdot}2 \pm 32{\cdot}3\%$, $n = 4$) demonstrated any significant change in the natural killer cell activity.

The effects of the MPO stimulation on the efferent discharges of the splenic nerve

Bilateral stimulation of the MPO decreased the efferent discharge rate of the splenic nerve in all the six rats examined. As shown in Fig. 1*A*, the stimulation ranged from 0·1 to 0·3 mA at 100 Hz produced an intensity-dependent suppression of the nerve activity. The firing rate decreased from a prestimulation level of $82{\cdot}6 \pm 9{\cdot}5$ impulses/2 s (means $\pm$ S.D.) to $52{\cdot}3 \pm 5{\cdot}5$ impulses/2 s (-37%) at 0·3 mA. The inhibition recovered to the baseline level immediately after the cessation of stimulation except for the suppression induced by 0·3 mA stimulation which showed a slow recovery of the nerve activity. There was no apparent difference among the effects of the MPO stimulation of the same intensity (0·2 mA) at different frequencies (5–100 Hz) (Fig. 1*B*).

Since the reduction of the nerve activity was always accompanied by a transient decrease in the systemic blood pressure (Fig. 1*A* and *B*), the effect of changes in blood pressure on the nerve activity was investigated. When the blood pressure rose following an I.V. injection of a peripheral acting vasoconstrictor, phenylephrine (3 μg/0·3 ml), the splenic nerve showed a marked decrease in activity, which was followed by a rebound increase (Fig. 1*C*).

The effects of the MPO lesion on the efferent activity of the splenic nerve

Electrical coagulation of the MPO produced a sustained rise in the nerve activity of about 1·5–2 times the baseline level in all six animals examined (Fig. 2). The nerve activity was enhanced a few minutes after the lesion was made and lasted several hours (from $88{\cdot}9 \pm 8{\cdot}6$ impulses/5 s to $152{\cdot}2 \pm 7{\cdot}5$ impulses/5 s, $+71\%$). A transient and marked decreased in the nerve activity observed immediately after the start of

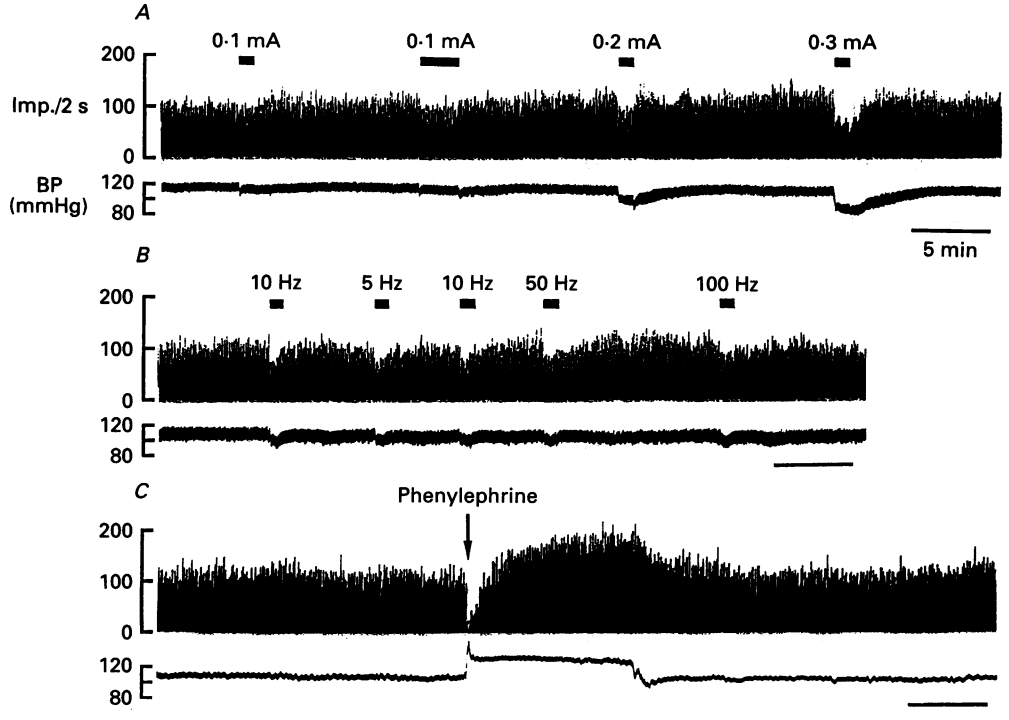

Fig. 1. The effects of electrical stimulation of MPO and I.V. injection of phenylephrine on the efferent discharge rates of splenic nerve filaments. *A*, the filled horizontal bars indicate the duration of bilateral stimulation of the MPO (0·1 ms duration, rectangular pulses). The intensity is shown above each bar and frequency is 100 Hz except for the first stimulation (50 Hz). *B*, filled horizontal bars, duration of bilateral stimulation of the MPO (0·1 ms duration, 0·2 mA intensity) at frequencies of 5–100 Hz as indicated above each bar. *C*, effects of I.V. injection of phenylephrine (3 μg/0·3 ml) indicated by an arrow. Lower traces of *A*, *B* and *C*, systemic blood pressure.

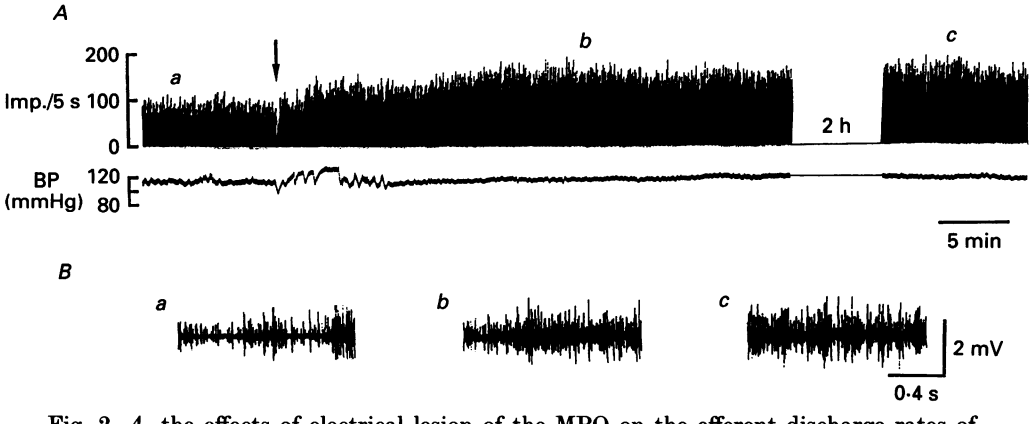

Fig. 2. *A*, the effects of electrical lesion of the MPO on the efferent discharge rates of splenic nerve filaments. An arrow indicates the time when the bilateral lesion of the MPO was made by passing a 1 mA direct current for 15 s. Lower trace, systemic blood pressure. *Ba*, *b*, and *c*, multiple unit discharges sampled at the corresponding time in the upper trace, respectively.

direct current application may be the result of the intense stimulation of the MPO by the direct current. Although the systemic blood pressure fluctuated for several minutes after the MPO lesion, it became stable until the end of the experiment (Fig. 2).

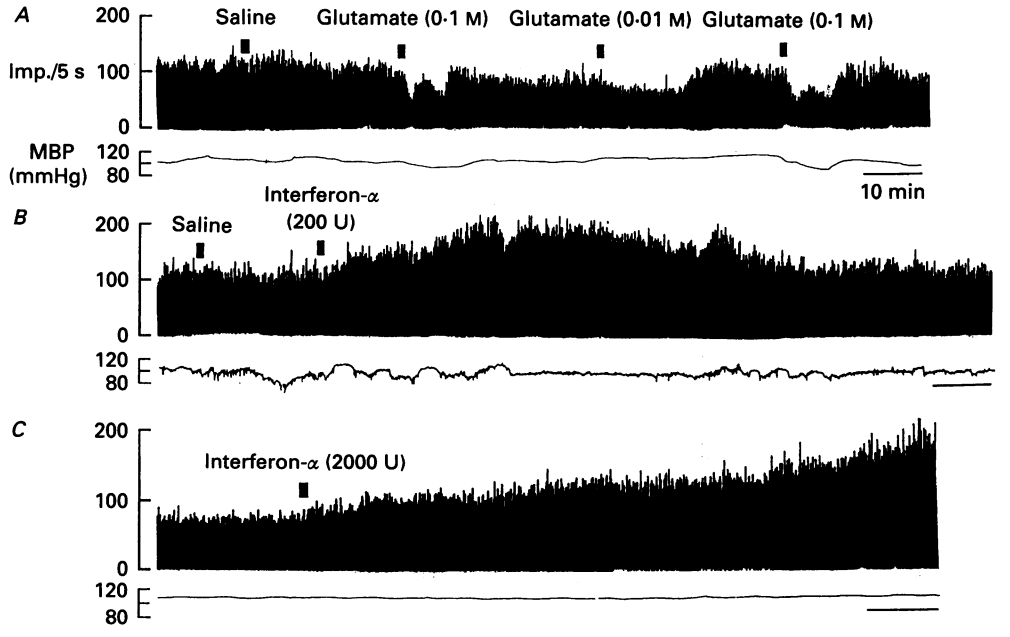

Fig. 3. The effects of microinjection of glutamate and interferon-α into the MPO on the efferent discharge rates of splenic nerve filaments. *A*, *B* and *C*, different preparations. Filled bars indicate the duration of microinjection of glutamate, interferon-α and saline. Drugs were dissolved in 0·1 μl saline at the concentrations indicated in parentheses. Lower traces of *A*, *B* and *C*, mean arterial blood pressure.

The effects of chemical stimulation of the MPO on the efferent activity of the splenic nerve

Microinjection of glutamate (0·1 M in 0·1 μl saline) into the MPO produced a suppression of the splenic nerve activity ($n = 3$) within 1 min of the beginning of the infusion, which lasted for about 10 min (Fig. 3*A*). The nerve activity decreased from a pre-infusion level of 94·3 $\pm$ 5·4 impulses/5 s to 52·7 $\pm$ 7·9 impulses/5 s (−44%). An injection of 0·01 M glutamate also induced a slight inhibition of the nerve activity, whereas an injection of saline (0·1 μl) had no effect. The hypotension was observed depending on the dose of glutamate.

On the other hand, microinjection of interferon-α at a dose of 200 U/0·1 μl saline into the MPO produced an increase in the nerve activity after a latency of about 1 min which lasted for about 1 h (Fig. 3*B*, from 98·8 $\pm$ 9·9 impulses/5 s to 182·5 $\pm$ 10·1 impulses/5 s, +85%). In this case, the mean arterial blood pressure showed a fluctuation which was not associated with the changes in the nerve activity. An injection of a larger dose of interferon-α (2000 U/0·1 μl saline) caused a more

pronounced response which lasted at least 2 h (Fig. 3*C*, 72·1 ± 4·8 impulses/5 s to 188·5 ± 9·0 impulses/5 s, ± 161 %).

Summary of the locations of the electrical and chemical stimulations

Fig. 4 summarizes the location of brain sites which received the electrical and chemical stimulations and the responses of the splenic nerve activity. The suppression

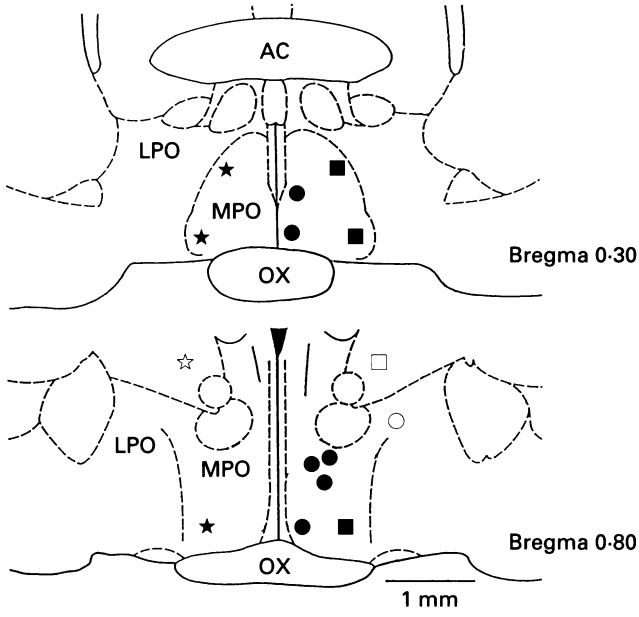

Fig. 4. The locations of the brain sites which received electrical and chemical stimulations. ●, sites where inhibitory responses to electrical stimulation were evoked ($n = 6$). ○, a site where no response occurred. Although the electrical stimulation was done bilaterally, only the unilateral sites were illustrated. ■, inhibitory responses to microinjection of glutamate (0·1 M in 0·1 μl saline). ★, excitatory responses to microinjection of interferon-α (200 U in 0·1 μl saline). □ and ☆, no change. Although glutamate and interferon-α were injected unilaterally through the same cannula, the sites of infusion of two substances were plotted on the opposite side, with each other. MPO, medial part of the preoptic hypothalamus; LPO, lateral part of the preoptic hypothalamus; AC, anterior commissure; OX, optic chiasma. Distance from bregma of each section is 0·3 and 0·8 mm, respectively.

of the nerve activity was consistently evoked when the tips of the stimulation electrodes were correctly located in the MPO ($n = 6$, ●), but not when they were found outside the MPO ($n = 1$, ○). The tips of the bilateral stimulation electrodes were found almost symmetrically with respect to mid-line.

Histological examinations revealed that the maximal diameters of the spread of injected solutions, which were judged as blue spots stained by Pontamine Sky Blue, were less than 0·5 mm. All the cannulae for the microinjections of glutamate and interferon-α that produced a decrease and increase in the nerve activity, respectively, were correctly located in the MPO. Only one animal, in which the tip of the cannula was outside the MPO, showed no alteration of the nerve activity after injections of either glutamate or interferon-α.

The effects of chemical stimulation of the PVN on the splenic nerve activity

A microinjection of glutamate (0·01 M/0·1 μl saline) into the PVN produced a sustained facilitation of the splenic nerve activity for more than 1 h, whereas an infusion of the vehicle (saline) had no effect at all in three animals examined. In Fig.

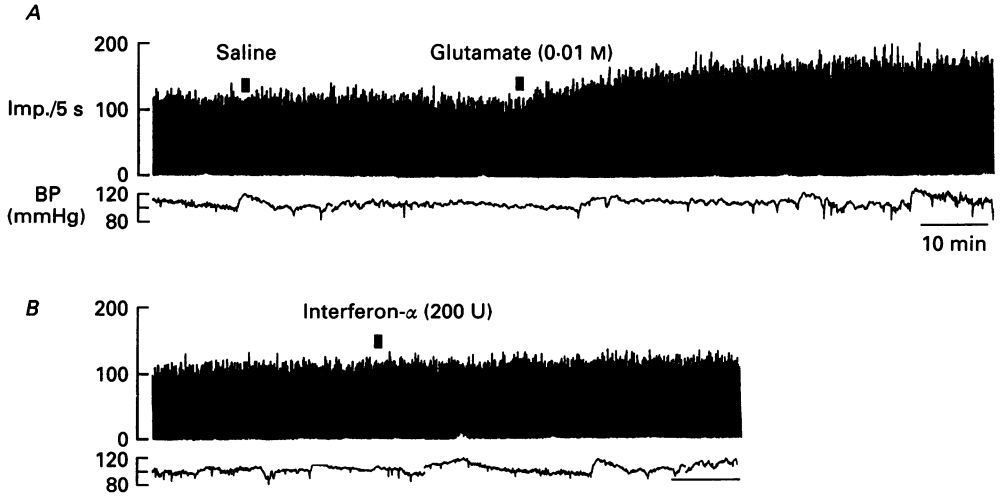

Fig. 5. The effects of microinjection of glutamate and interferon-α into the PVN on the efferent discharge rates of the splenic nerve filaments. *A* and *B*, different preparations. Filled bars indicate the duration of microinjection of glutamate, interferon-α and saline. Drugs were dissolved in 0·1 μl saline at each concentration indicated in parentheses. Lower traces of *A* and *B*, mean arterial blood pressure.

5*A*, the nerve activity increased from a pre-infusion level of 108·6 $\pm$ 6·6 impulses/5 s to 180·1 $\pm$ 8·2 impulses/5 s (+66%). However, a microinjection of interferon-α (200 and 2000 U in 0·1 μl saline) into the PVN was without effect in all three rats (Fig. 5*B*). Histological studies revealed that the blue spots made by 0·1 μl dye injection in these animals were confined within the PVN.

DISCUSSION

Involvement of the splenic nerve in the MPO lesion-induced suppression of the splenic natural killer cell activity

Several reports have indicated that lesions of the MPO result in a suppression of peripheral immunity as reflected by changes in antibody production, delayed hypersensitivity, lymphocyte mitogenesis and cytotoxic activity of splenic natural killer cells (Cross *et al.* 1982, 1984; Jankovic & Spector, 1986). It has been demonstrated that the splenic natural killer cell activity of Fischer 344 rats was reduced 4 and 7 days after the ablation of the MPO, but not after lesion of the frontal cortex, it then recovered to normal by day 14 (Cross *et al.* 1984), and it has also been shown that this effect of the hypothalamic lesion was not a result of increased circulating corticosterone levels (Cross, Markesbery, Brooks & Roszman, 1980). Since

there was no difference in natural killer cell activity after hypophysectomy between MPO-lesioned rats and control rats (Cross *et al.* 1984), it can be concluded that pituitary hormones were involved in the reduced cytotoxicity of the natural killer cells. However, hypophysectomy itself suppressed the splenic natural killer cell activity in frontal cortex-lesioned rats although such lesions were without effect when the hypophysis was intact (Cross *et al.* 1984). Therefore, it is possible to suggest that the hypophysectomized animals would not further reduce the natural killer cell activity after the MPO lesion.

In the present study, the cytotoxic activity of the natural killer cells in the spleen was reduced 20 min after lesioning the MPO. It has been reported that the levels of serum corticosterone are elevated 1 h after the lesioning of either the MPO or the frontal cortex (Cross *et al.* 1984). However, our results showed no change in the natural killer cell activity after thalamic lesion, which would be expected to similarly raise the steroid level. Since the suppression was completely blocked by surgical denervation of the splenic nerve, we conclude that the splenic nerve plays a crucial role in mediating the reduction of the natural killer cell activity observed 20 min after ablation of the MPO.

The splenic nerve activity and the cytotoxicity of the natural killer cells

There is good evidence that the sympathetic nervous system may be involved in the control of cellular immunity in the spleen (see review by Madden & Livnat, 1991). The splenic nerve innervates not only the blood vessels but also the parenchymal tissues near the periarteriolar lymphatic sheath and parafollicular or marginal zones. Electron microscopic observations have revealed the presence of synaptic-like contacts between tyrosine hydroxylase-positive nerve terminals and the splenic lymphocytes (Felten *et al.* 1987). The splenic natural killer cell activity is enhanced by splenic denervation (Reder, Checinski & Chelmicka-Schorr, 1989) and is suppressed by exposure to noradrenaline or its agonist *in vitro* (Hellstrand, Hermodsson & Strannegård, 1985). Furthermore, we recently found that electrical stimulation of the splenic nerve for 20 min resulted in a suppression of the splenic natural killer cell activity, which was blocked by an I.V. injection of a peripherally acting β-adrenergic blocking agent, nadolol, indicating a β-adrenergic receptor-mediated suppression of the splenic natural killer cell activity (Katafuchi *et al.* 1993).

The present study revealed that the bilateral lesion of the MPO produced a decrease in the cytotoxic activity of the splenic natural killer cells, which was dependent on the intact splenic nerve and was accompanied by the long-lasting increase in the splenic nerve activity (Fig. 2). These findings and the previous ones, taken together, suggest that the suppression of the splenic natural killer cell activity after the MPO lesion is mediated by the increased release of noradrenaline from the splenic nerve terminals.

The influences of the MPO on the efferent discharges of the splenic nerve

Electrical stimulation of the bilateral MPO inhibited the efferent discharge rates of the splenic nerve in an intensity-dependent manner (Fig. 1*A*). Since the activity of the splenic sympathetic nerve was shown to be inhibited by inputs from the baroreceptors (Ninomiya, Nisimaru & Irisawa, 1971), a marked suppression of the

nerve activity observed immediately after an I.V. injection of phenylephrine (Fig. 1*C*) was thought to be a result of an acute elevation of blood pressure. However, since the suppression of the splenic nerve activity during MPO stimulation was accompanied by a fall in systemic blood pressure (Fig. 1*A* and *B*), the hypotension itself cannot explain the observed reduction of the nerve activity. In contrast to the effects of MPO stimulation, bilateral lesion of the MPO resulted in a sustained increase in nerve activity (Fig. 2), suggesting that the MPO has an inhibitory influence on the splenic sympathetic nerve activity. This conclusion is also supported by the observation that microinjection of glutamate into the MPO, which was thought to activate neuronal cell bodies but not the passing fibres, produced a transient suppression of the splenic nerve activity (Fig. 3*A*).

Involvement of the MPO in the interferon-α-induced suppression of the splenic natural killer cell activity

Intraventricular injection of interferon-α facilitates the electrical activity of the splenic nerve (Katafuchi *et al.* 1991; Katafuchi *et al.* 1993). Administration of interferon-α (1000–20000 U) into the lateral cerebroventricle resulted in the suppression of the cytotoxicity of the splenic natural killer cells which depends on the intact innervation of the splenic nerve (Hori *et al.* 1991*b*). The present study demonstrated that only 200 U of interferon-α into the MPO (Fig. 3), but not into the PVN (Fig. 5), was effective in increasing the splenic nerve activity to the same degree as that observed after the intra-third ventricular injection at a dose of 1500 U in rats (Katafuchi *et al.* 1993). Furthermore, we have found that the microinjection of 200 U interferon-α into the MPO reduced the splenic natural killer cell activity to the same degree as that observed after lateral cerebroventricular injection at a dose of 20000 U in rats (Take *et al.* 1992*b*). These findings suggest that the MPO may be one site of action of centrally administered interferon-α.

About 51% of the MPO neurones in tissue slice preparations are inhibited following direct application of interferon-α (Nakashima, Hori, Kuriyama & Matsuda, 1988). Since the MPO has an inhibitory influence on the splenic nerve activity, the facilitation of the nerve activity following an injection of interferon-α into the MPO may be caused by a reduction of the inhibitory control (disinhibition) of the MPO on the splenic sympathetic nerve.

Regulation of the splenic natural killer cell activity by the hypothalamo-sympathetic nervous system

The hypothalamus is thought to be involved in the modulation of visceral autonomic nerve activities (Oomura & Yoshimatsu, 1984). There are monosynaptic and polysynaptic projections from the hypothalamus to the sympathetic preganglionic neurones in the spinal cord (Saper, Loewy, Swanson & Cowan, 1976; Palkovits & Zárborszky, 1979), but the changes in the splenic nerve activity produced by microinjection of interferon-α into the MPO might involve connections between the MPO and other hypothalamic regions. There is a direct projection from the MPO to the PVN (Conrad & Pfaff, 1975; Swanson, 1976) and electrical stimulation of the MPO has been reported to suppress the multiple unit activity of the PVN in freely moving rats (Saphier & Feldman, 1986). The present results

indicate that the PVN has an excitatory influence on the splenic nerve activity since microinjection of glutamate into the PVN increased it (Fig. 5). Therefore, injection of interferon-α into the MPO, which suppresses the MPO neurones (Nakashima *et al.* 1988), may lead to a disinhibition of the activity of the PVN neurones, thereby producing the enhancement of the splenic nerve activity.

The splenic sympathetic nerve constitutes one of the communication channels that mediate the central modulation of peripheral cellular immunity (Katafuchi *et al.* 1993). The suppression of the splenic natural killer cell activity induced by central injection of interferon-α (Take *et al.* 1992*b*), interleukin 1-β (Sundar, Cierpial, Kilts, Ritchie & Weiss, 1990), and corticotropin-releasing factor (Irwin, Hauger, Brown & Britton, 1988; Hori *et al.* 1991*b*) were shown to be completely or partially blocked by either an administration of a ganglionic blocker or a surgical denervation of the splenic nerve. The present results add further evidence for the importance of the hypothalamo-sympathetic nervous system in the neural control of the immune system.

We thank Dr B. T. Quinn, Kyushu University for reading this manuscript. This study was supported by Special Coordination Funds of the Science and Technology Agency of the Japanese Government (to T.H.). This work was also supported in part by Grants-in-Aid for Scientific Research 03670079 (to T.K.), 03557009 and 04454144 (to T.H.) from the Ministry of Education, Science, and Culture, Japan and a grant provided by The Naito Foundation (to T.K.).

REFERENCES

BELLINGER, D. L., FELTEN, S. Y., LORTON, D. & FELTEN, D. L. (1989). Origin of noradrenergic innervation of the spleen in rats. *Brain, Behavior, and Immunity* **3**, 291–311.

CONRAD, L. C. A. & PFAFF, D. W. (1975). Axonal projections of medial preoptic and anterior hypothalamic neurons. *Science* **190**, 1112–1114.

CROSS, R. J., BROOKS, W. H., ROSZMAN, T. L. & MARKESBERY, W. R. (1982). Hypothalamic-immune interactions. Effect of hypophysectomy on neuroimmunomodulation. *Journal of Neurological Sciences* **53**, 557–566.

CROSS, R. J., MARKESBERY, W. R., BROOKS, W. H. & ROSZMAN, T. L. (1980). Hypothalamic-immune interactions. I. The acute effect of anterior hypothalamic lesions on the immune response. *Brain Research* **196**, 79–87.

CROSS, R. J., MARKESBERY, W. R., BROOKS, W. H. & ROSZMAN, T. L. (1984). Hypothalamic-immune interactions: neuromodulation of natural killer activity by lesioning of the anterior hypothalamus. *Immunology* **51**, 399–405.

FELTEN, D. L., FELTEN, S. Y., BELLINGER, D. L., CARLSON, S. L., ACKERMAN, K. D., MADDEN, K. S., OLSCHOWKI, J. A. & LIVNAT, S. (1987). Noradrenergic sympathetic neural interactions with the immune system: structure and function. *Immunological Reviews* **100**, 225–260.

FELTEN, S. Y. & FELTEN, D. L. (1991). Innervation of lymphoid tissue. In *Psychoneuroimmunology*, ed. ADER, R., FELTEN, D. L. & COHEN, N., pp. 27–61. Academic Press, New York.

HELLSTRAND, K., HERMODSSON, S. & STRANNEGÅRD, Ö. (1985). Evidence for a β-adrenoceptor-mediated regulation of human natural killer cells. *Journal of Immunology* **134**, 4095–4099.

HORI, T., NAKASHIMA, T., TAKE, S., KAIZUKA, Y., MORI, T. & KATAFUCHI, T. (1991*a*). Immune cytokines and regulation of body temperature, food intake and cellular immunity. *Brain Research Bulletin* **27**, 309–313.

HORI, T., TAKE, S., MORI, T., KAIZUKA, Y., SHIMIZU, N. & KATAFUCHI, T. (1991*b*). Mechanisms of central opioids-induced suppression of rat peripheral cellular immunity. *Society for Neuroscience Abstracts* **17**, 328.14, 833.

IRWIN, M., HAUGER, R. L., BROWN, M. & BRITTON, K. T. (1988). CRF activates autonomic nervous system and reduces natural killer cytotoxicity. *American Journal of Physiology* **255**, R744–747.

JANKOVIĆ, B. D. & SPECTOR, N. H. (1986). Effects on the immune system of lesioning and stimulation of the nervous system: neuroimmunodulation. In *Enkephalins and Endorphins, Stress and the Immune System*, ed. PLOTNIKOFF, N. P., FAITH, R. E., MURGO, A. J. & GOOD, R. A., pp. 189–220. Plenum Press, New York.

KATAFUCHI, T., HORI, T. & TAKE, S. (1991). Central administration of interferon-α enhances rat sympathetic nerve activity to the spleen. *Neuroscience Letters* **125**, 37–40.

KATAFUCHI, T., TAKE, S. & HORI, T. (1993). Roles of sympathetic nervous system in the suppression of cytotoxicity of splenic natural killer cells in the rat. *Journal of Physiology* **465**, 343–357.

KATAFUCHI, T., TAKE, S., ICHIJO, T. & HORI, T. (1992). Hypothalamic control of peripheral immune functions mediated by the sympathetic nervous system in rats. *Society for Neuroscience Abstracts* **18**, 288.11, 681.

LABEUR, M., NAHMOD, V. E., FINKIELMAN, S. & ARZT, E. (1991). Lesions of the medial septal nucleus produce a long-lasting inhibition of T lymphocyte proliferation. *Neuroscience Letters* **125**, 129–132.

MADDEN, K. S. & LIVNAT, S. (1991). Catecholamine action and immunologic reactivity. In *Psychoneuroimmunology*, ed. ADER, R., FELTEN, D. L. & COHEN, N., pp. 283–310. Academic Press, New York.

NAKASHIMA, T., HORI, T., KURIYAMA, K. & MATSUDA, T. (1988). Effects of interferon-α on the activity of preoptic thermosensitive neurons in tissue slices. *Brain Research* **454**, 361–367.

NANCE, D. M. & BURNS, J. (1989). Innervation of the spleen in the rat: evidence for absence of afferent innervation. *Brain, Behavior, and Immunity* **3**, 281–290.

NINOMIYA, I., NISIMARU, N. & IRISAWA, H. (1971). Sympathetic nerve activity to the spleen, kidney, and heart in response to baroreceptor input. *American Journal of Physiology* **221**, 1346–1351.

OOMURA, Y. & YOSHIMATSU, H. (1984). Neural network of glucose monitoring system. *Journal of Autonomic Nervous System* **10**, 359–372.

PALKOVITS, M. & ZÁBORSZKY, L. (1979). Neural connections of the hypothalamus. In *Handbook of the Hypothalamus*, vol. 1, *Anatomy of the Hypothalamus*, ed. MORGANE, P. J. & PANKSEPP, J., pp. 379–509. Marcel Dekker, New York.

PAXINOS, G. & WATSON, C. (1986). *The Rat Brain in Stereotaxic Coordinates*. Academic Press, New York.

REDER, A., CHECINSKI, M. & CHELMICKA-SCHORR, E. (1989). The effect of chemical sympathectomy on natural killer cells in mice. *Brain, Behavior, and Immunity* **3**, 110–118.

ROSZMAN, T. L., CROSS, R. J., BROOKS, W. H. & MARKESBERY, W. R. (1985). Neuroimmunodulation: effects of neural lesions on cellular immunity. In *Neural Modulation of Immunity*, ed. GUILLEMIN, R., COHN, M. & MELNECHUK, T., pp. 95–109. Raven Press, New York.

SAPER, C. B., LOEWY, A. D., SWANSON, L. W. & COWAN, W. M. (1976). Direct hypothalamo-autonomic connections. *Brain Research* **117**, 305–312.

SAPHIER, D. & FELDMAN, S. (1986). Effects of stimulation of the preoptic area on hypothalamic paraventricular nucleus unit activity and corticosterone secretion in freely moving rats. *Neuroendocrinology* **42**, 167–173.

SUNDAR, S. K., CIERPIAL, M. A., KILTS, C., RITCHIE, J. C. & WEISS, J. M. (1990). Brain IL-1-induced immunosuppression occurs through activation of both pituitary-adrenal axis and sympathetic nervous system by corticotropin-releasing factor. *Journal of Neuroscience* **10**, 3701–3706.

SWANSON, L. W. (1976). An autoradiographic study of the efferent connections of the preoptic region in the rat. *Journal of Comparative Neurology* **167**, 227–256.

TAKE, S., MORI, T., KATAFUCHI, T., KAIZUKA, Y. & HORI, T. (1992*a*). Central interferon α suppresses the cytotoxic activity of natural killer cells in the mouse spleen. *Annals of the New York Academy of Sciences* **650**, 46–50.

TAKE, S., KATAFUCHI, T., ANDO, T., UCHIMURA, D., KANEMITSU, Y., ICHIJO, T., SHIMIZU, N., HORI, T. & KOSAKA, T. (1992*b*). Hypothalamic interferon α (IFNα) reduces splenic NK cytotoxicity. *Society for Neuroscience Abstracts* **18**, 288.12, 681.