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NEUROIMMUNOENDOCRINOLOGY OF THE CERVICAL AUTO NO MIC NERVOUS SYSTEM

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SUMMARY

• *This article reviews several peripheral neuroendocrine relationships in the cervical region, namely the effect of the sympathetic innervation on adenohypophysial, thyroid and parathyroid hormone release, and the effect ofparasympathetic innervation in thyroid and parathyroid glands. The possible pathways through which the central nervous system modulates the circadian organization of the immune response are also reviewed and the relative importance of circadian control of immune reactivity through local sympathetic and parasympathetic nerves and of newoendocrine signals, like melatonin, is also discussed. Altogether the present article argues in favor of the concept that nerves arriving to the endocrine and lymphoid tissue constitute alternate pathways through which the brain controls immunoendocrine phenom ena. (**BiomedRev 1998; 9:** 47-59)*

INTRODUCTION

• Besides affecting the contraction of a skeletal or smo oth muscle, the autonomic motor leg of the peripheral nervous system directly modulates exocrine, endocrine, and paracrine relationships in secretory cells. Through the innervation of lymphoid tissues and blood marrow, the autonomic nervous system affects the amplitude of proliferative responses during the immune reaction or after a reduction of the erythrocyte mass. There is indeed an increasing information on the importance of appropriate sympathetic and parasympathetic local environments for a correct communication among proliferative cells. In turn, the activity of sensory fibers, e.g. substance P-containing fibers, in the vicinity of endocrine and immune cells may serve to build up in the brain a viscerotopic map not dissimilar from that of dermatomes in the somatic nervous system. This may subserve a continuous monitoring of the neuro-endocrineimmune "posture", feeding back to the unconscious part of the brain.

The particular endocrine significance of the motor autonomic projections has been suspected since long. As an example of such an early interest, physiologists in the early thirties entertained several possible autonomic motor mechanisms to explain the ovulatory response that followed the cephalic electrical stimulation in rabbits that included communication to the adenohypophysis *via* the greater superficial petrosal nerve and the sphenopalatine ganglion, the cervical sympathetic chain, and the hypophyseal stalk (1-3). Although eventually the vascular connection derived from the pituitary stalk became the subject of central attention, a vascular adenohypophyseal innervation derived from sympathetic superior cervical ganglia (SCO) was described anatomically (4,5) and several endocrine sequela of SCO or sphenopalatine or vidian ganglia ablation were reported in rats (1-3).

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Our interest in the neuroendocrine consequences of the manipulation of the peripheral sympathetic projections to the adenohypophysis arose from studies on the physiology of the neural input to the pineal gland, an organ heavily depending on SCO projections to synthesize the hormone melatonin. In a number of experiments we, as well as other researchers, found that pinealectomy (Px) and superior cervical ganglionectomy (SCGx), a procedure supposed to act exclusively *via* suppression of melatonin synthesis, differed in their consequences for such disparate behaviors as regulation of prolactin (PRL) release, of circadian rhythms or of water or sodium appetite (6-10).

The present review article summarizes data indicating a functional regulation of medial basal hypothalamus (MBH) by SCO projections. How peripheral autonomic nerves of the cervical region modulate activity in endocrine organs (thyroid and parathyroid glands) and immune tissue (the submaxillary lymph nodes) is also reviewed. Finally, the relative involvement of the autonomic nervous system and of hormones like melatonin to convey circadian signals to the irnmunocompetent cells is discussed. Previous publications provide with detail different aspects of the subjects herein described (2,3,11-13).

AUTONOMIC PROJECTIONS IM THE CERVICAL REGION

• A number of neuroimmunoendocrine relevant structures are located in the cervical-cephalic region, among them the MBH-hypophyseal complex, thyroid and parathyroid glands, primary and secondary lymph organs, e.g. the thymus and the submaxillary lymph nodes, respectively, as well as the pineal and submandibular glands (2,3,11,12,14). As it is the case for many other autonomic territories, three major neuronal systems provide innervation to endocrine and immune structures in the cervical region. They comprise of *(i)* sympathetic noradrenergic neurons, located in the superior, inferior and/or middle cervical ganglia and reaching the organs *via* the SCO and the external carotid nerve (15,16), *(ii)* parasympathetic cholinergic neurons located in regional ganglia and receiving preganglionic input from the vagus nerve, and *(Hi)* peptidergic neurons, most of them belonging to local ganglia (17) (Fig. 1).

Axons leaving the SCG provide sympathetic innervation to facial structures, the skull and the neck. In the rat, SCG ganglio-nic neurons are grouped in three main associations, including *(i)* a rostral group that projects *via* the internal carotid nerve (about 35% of SCG neurons), *(ii)* a caudal group that projects *via the* external carotid nerve (about 50% of SCG neurons), and *(Hi)* a caudal group that sends descendent projections through the cervical sympathetic trunk (about 15% of SCG neurons). Topographical studies of functional subpopulations of neurons in the rat SCG (18) indicated that individual neurons have very limited projection fields and minimal contralateral innervation of bilateral targets. There was a general rostrocaudal organization of neurons with respect to the position of their targets and it con-elated with the exit sites of the neurons from the ganglion. The pineal gland, for example, is innervated by

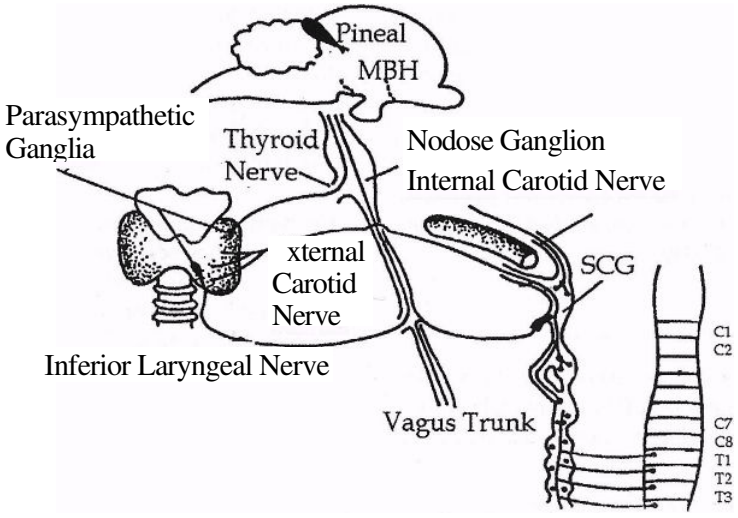

Figure 1. *Autonomic innervation of thyroid and parathyroid glands in the cervical region. The three top ganglia of the sympathetic chain, i.e. SCG, medial and inferior cervical ganglia, as well as the nodose ganglion in the vagus nerve trunk, are depicted.*

an estimated 4% of rostrally located SCO neurons which reach the gland *via* the internal carotid nerve. The internal carotid nerve pathway is also the pathway followed by SCG postganglionic fibers innervating intracranial structures such as the MBH or the neurohypophysis (Fig. 1). A peculiarity of the thyroid and parathyroid gland innervation provided by the SCG is that some of the innervating ganglionic perikarya are located in the middle and/or inferior sympathetic cervical ganglia and send their axons through the SCG and the external carotid nerves (15,16). Another discrete population of innervating thyroid sympathetic neurons is located in the SCG, as shown by retrograde tracing and immunocytochemistry. Several strategies have been employed to examine the neuroendocrine consequences of autonomic nerve manipulation. In some cases, a "deprivation experiment" was performed by examining the endocrine sequelae of SCGx or of parasympametic decentralization 14 weeks after surgery. In other cases, the transient postsynaptic activation that occurs either after electrical stimulation of the cervical sympathetic trunk to the SCG (19,20) or during the early phase of anterograde (Wallerian) degeneration of the sympathetic nerve endings in SCGx animals was analyzed (degeneration reaction) (21). Concerning Wallerian degeneration of nerves after SCGx, it is well established that, with a latency of 12 days, section of an adrenergic nerve is followed by a transient hyperactivity of the denervated organ as a consequence of the supraliminal transmitter release from degenerating adrenergic varicosities (22). One of the advantages of the degeneration activity paradigm is that the full period of Wallerian degeneration in the SCG pathway can be readily monitored in this way in the conscious, undisturbed animal by the extent of palpebral retraction (due to degeneration activity at the level of the periorbital muscles). It should however be noted that the degeneration-induced, continuous transmitter release is only an approximate indication of the phasic, physiologic release of transmitter that follows the stimulation of the afferent pathway. In the case of chronic denervation studies, the surgical denervations have clearly more advantages than, for example, a general chemical sympathectomy obtained by injecting the adrenergic neurotoxin 6-hydroxydopamine. Indeed, the general toxic effects of the drug can never be ruled out, and a number of autonomic systemic reflex sequelae arise, making it difficult to interpret the results obtained.

PROJECTIONS OF SUPERIOR CERVICAL GANGLIA TO MEDIAL BASAL HYPOTHALAMUS

• The notion that norepinephrin (NE)-containing terminals in the hypothalamus are mostly projections from cell bodies in the brainstem is accepted undisputedly (23). However, a peripheral source of basal hypothalamic NE is demonstrable by several means. For example, early histochemical fluorescen ce studies have indicated that after SCGx, the large, coarse, catecholamine-containing fibers associated primarily with blood vessels in the neurointermediate lobe disappear (24) and several years ago we reported a significant decrease of MBH 3H-NE uptake (25) and a 40-60% decrease of rat median eminence NE content after SCGx (26). Such drastic changes in transmitter high affinity uptake and content indicated that at least a portion of noradrenergic nerve terminals in the median eminence derived from the SCG. Indeed, the concentration of NE in rat median eminence did not fall to zero after bilateral 6hydroxydopamine lesions of the ventral pons, suggesting that as much as 25-40% of the NE in the median eminence originates in anatomical structures outside the ventral bundle (27). To obtain information on other possible neurochemical sequela of SCGx in MBH, we examined recently the metabolism of dopamine and serotonin (28). Eight days after SCGx of rats, MBH dopamine and serotonin turnover decreased, while the steady state levels of biogenic amines increased, indicating that peripheral sympathetic denervation of MBH may lead to a depression of dopamine and serotonin release. Moreover, MBH changes caused by the ectopic PRL secretion from a pituitary graft, namely the increase of dopamine turnover and decrease of serotonin turnover, were attenuated by SCGx (28). Other areas anatomically related to the median eminence become partially - denervated after SCGx. This is the case of the neurointermediate lobe, whose NE fibers are in part peripheral, as shown by the 40% decrease of posterior pituitary NE content after SCGx (29). a 1-adrenoceptor binding sites, autoradiographically detectable in the neural lobe of the rat pituitary, increased in number after SCGx, but not after pituitary stalktransection, thus indicating their association with peripheral NE-containing fibers (30).

In order to show that the neurochemical changes in MBH during the degeneration phase of sympathetic nerve endings after SCGx were accompanied by modifications of adenohypophyseal hormone release, a number of experiments were carried out, largely in our laboratory, during the last 15 years (2,11,12). As far as the hypothalamic-hypophyseal-ovarian axis, SCGx of cycling female rats, performed at 24:00 h of diestrus II, i.e. about 16-18 hours in advance of the critical period of gonadotropin and PRL release, caused 14 days delay of cycle, as shown by leukocytic diestrous vaginal smears, at the end of which the estrous cycle resumed. SCGx at 24:00 h on the day of estrus did not modify estrous cyclicity (31). The results indicated that sympathetic nerve degeneration after SCGx must coincide with the stimulus for gonadotropin release, e.g. its preovulatory surge, to disrupt estrous cyclicity. The inhibitory effect of peripheral Wallerian degeneration on gonadotropin and PRL release was also found in Px rats (31), indicating that the effect observed was independent of the melatonin release taking place during Wallerian degeneration. In confirmation of the cycling in rats mentioned above. Dziedzic and Walczewska (32) recently reported that SCGx decreased luteinizing hormone-releasing hormone (LHRH) content in the median eminence of female rats when the surgery was performed 12 h, but not 48 h, in ad-

vance to sacrifice. It is interesting that under certain circum stances, like the post-orchidectomy rise of luteinizing hormone (LH), the MBH concentration of LHRH increased, while the pituitary response to LHRH remained normal, at the time of Wallerian degeneration of sympathetic nerves after SCGx (33). Collectively, the observations suggest that a major effect of peripheral nerve endings in the median eminence is the modu lation of LHRH release.

In the case of hypothalamic-hypophyseal-thyroid axis, we, as well as others, found that a significant depression of thyroid-stimulating hormone (TSH) release taking place during the anterograde degeneration phase after SCGx (21,34). Similar to what was found for the LHRH response, a normal pituitary response to thyrotropin releasing hormone (TRH) was observed during the Wallerian degeneration of sympathetic nerves. In a study designed to examine the levels of serum growth hormone (GH) and PRL, and of MBH GH-releasing hormone (GHRH), thyrotropin-releasing hormone (TRH) and somato-statin, from 16 h to 7 days after bilateral SCGx of adult male rats (35) we reported significantly lower serum GH and PRL, and higher MBH GHRH and TRH levels as compared to controls during the Wallerian degeneration phase; MBH somatostatin concentration decreased in SCGx rats. The data, together with the previously observed decrease in MBH corticotropin-releasing hormone (CRH) and arginine vasopressin (AVP) content found in SCGx rats during the Wallerian degeneration phase (36), as well as with the LHRH data mentioned above, indicated that a modulator/ role of peripheral sympathetic nerve terminals is exerted on hypophysiotropic hormone release. A previous Px did not modify the changes in hypophysiotropic or hypophyseal hormone release found during the Wallerian degeneration phase after SCGx. Some studies have also dealt with chronic effects of SCGx on adenohypophyseal hormone secretory mechanisms. Two weeks after SCGx, Tan and Ogawa (37), for example, reported that follicle-stimulating hormone (FSH) and LH cells of the rat hypophysis increased in number and immunostaining density, compatible with augmentation of their functional activity. On the contrary, GH cell number decreased. In the case of the hypothalamic-hypophyseal-adrenal axis, Siaud *et al* (38) reported that 5 to 10 days after SCGx, the circadian rhythms of ACTH and corticosterone were suppressed and the mean ACTH concentrations increased as compared to sham-operated rats, while the mean plasma corticosterone levels remained unmodified.

The observation of a depressed TSH release after acute SCGx led us to assess the homeostatic significance of SCO projections in the adaptation of rats to a cold environment. Regardless of the prior temperature at which the rats had been maintained, superimposing the anterograde degeneration of nerve terminals in the SCG field of projection to animals' exposure to a cold environment for 24 h, resulted in death of almost all SCGx rats, while sham-operated controls survived adequately (39). This was a strong indication that a normal SCG input is needed to produce an appropriate adaptive response to cold stress. We further examined the role of the SCG pathway in adaptation of rats to stress (40). By superimposing the Wallerian degeneration phase after SCGx to immobilization stress, an inhibition of the stress-induced LH and corticosterone response was found; the effect was not observed in animals submitted to SCGx 1 week earlier. In another study, the efficacy of a sympathetic thyroid denervation to prevent the thyroid axis depression evoked by an injection of the stressor agent turpentine oil in rats was assessed (41). We found that SCGx, or the bilateral section of external carotid nerves (the neural pathway connecting the SCG with the thyroid), but not of internal carotid nerves, prevented the depression of thyroid ^{131}I uptake or thyroxine (T4) release after turpentine. An increase in thyroid NE turnover rate was found in rats after turpentine oil injection
(41). Collectively, the above discussed results suggest that peripheral projections from the SCG play an important role in adaptation to stress.

As far as the neurointermediate lobe, data on changes of vasopressin release during Wallerian degeneration after SCGx (29) indicated a depressive effect of nerve degeneration, thus indicating a negative influence of peripheral sympathetic projections on neurohypophyseal hormone release. However, Lipinska *et al* (20) reported a vasopressin release, rather than inhibition, from posterior pituitary lobe incubated after acute preganglionic stimulation of the rat SCG, and in a recent study
(42) described an increase in vasopressin release into the hypophyseal portal blood after acute electrical SCG stimulation in the rat. Stimulation of SCG did not evoke an increase in oxytocin in the blood plasma of hypophyseal portal vessels, •> indicating the rather specific action of the peripheral sympa- i thetic projections. Other posterior pituitary lobe activities, like cardiodepressant activity, were also modified by the acute electrical stimulation of SCG nerves (43).

The reasons for the discrepancies between Wallerian degeneration and acute electrical stimulation of SCG studies as far as neurohypophyseal hormone release are not readily apparent; neither procedure provides the optimal physiological situation to analyze the involvement of SCG in neuroendocrine regulation. The continuous release of transmitter from degenerating nerves may cause changes in postsynaptic sensitivity that would lead to paradoxical results. On the other hand, a major drawback for the acute electrical stimulation paradigm of peripheral sympathetic nerves is that the animal must be immobilized and anesthetized, a situation which does interfere with the hormonal measurements. The development of programmed, unstressful nerve stimulation techniques in the conscious animal under long term basis could be very helpful to confirm and extend the data discussed herein.

The effect of adrenergic blocking agents on the changes observed during Wallerian degeneration after SCGx in rats has been thoroughly explored (2,11,12). Generally, degeneration activity was assoc iated with postsynaptic a 1 -adrenoceptor stimu lation, with some exceptions in which the effects were still observed following after a- plus (3-adrenergic blockade. To what extent neuropeptides, e.g. neuropeptide Y (NPY), are responsible for the nonadrenergic effects of acute SCGx needs to be explored. Indeed the pituitary gland receives both central and peripheral NPY innervation and within the posterior lobe of the pituitary gland two morphologically distinct NPY-immunoreactive fiber populations were discovered, namely thinner parenchymal terminals of a central origin, distinct from the neurosecretory terminals, and thicker, perivascular fibers, of SCG origin (44). The mechanism through which peripheral sympathetic neurons modify' median eminence function remain to be clarified. They may represent a particularly sensitive vasomotor effect of peripheral noradrenergic terminals. Alternatively, they may involve a more complex interrelationship, such as a modulatory effect on hypophysiotropic hormone release. Arterioles of human stalk and median eminence have highly muscular walls, and reflex constriction of these vessels following postpartum hemorrhage has been indicated as a factor in the genesis of postpartum pituitary infarction, Sheehan's syndrome (45). Indeed, a number of observations point out to the importance of SCG projections to regulate regional blood flow in brain. SCG activity affects the phenotype of smooth muscle cells of cerebral arteries (46) and is of great importance for cerebral blood flow autoregulation (47-49). SCG projections are one of the factors increasing the heterogeneity of venous oxygen saturation in selective brain regions (50) and the sympathetic innervation of the vasculature of the head plays role in the control of selective brain cooling of sheep (51). Indeed, SCGx resulted in a significant reduction of hypothalamic temperature. However, the hypophyseal portal blood flow did not change during preganglionic stimulation of SCG under condition of systemic arterial blood pressure stabilization in the rat (52).

AUTONOMIC INNERVATION OF THYROID GLAND ACINI

• A modulatory function of thyroid innervation on thyroid endocrine role has long been suspected (53). Auto nomic nerves arriving at the thyroid gland make contact not only with tissue vasculature but also with the endocrine cells, thus providing the anatomical basis for a peripheral neuroendocrine relationship (54,55) (Fig. 1).

• Effect of sympathetic innervation

It has been postulated that in the presence of very low or absent levels of TSH, catecholamines increase thyroid output, while in the presence of normal or augmented TSH levels such an action is inhibitory (54). In mice injected with NE and having suppressed or pharmacologically increased circulating TSH, changes in T4 are compatible with the proposed dual activity of NE. Experiments employing electrical stimulation of SCG in T4-treated mice have indicated a stimulatory activity of noradrenergic nerves in the presence of low or suppressed circulating TSH (54). On the other hand, injection of TSH into mice previously treated with 2-deoxyglucose, a drug that increases peripheral sympathetic activity, failed to augment T4 release (56). Likewise, we reported that in rats having degenerating sympathetic nerve terminals in the SCG field of projection after SCGx, the activity of exogenous TSH to increase T4 release was blunted (12). Therefore, in the presence of nearly normal or increased TSH levels, NE release from local sympathetic nerves provides a negative signal for T4 release.

Studies performed in chronically SCGx rats also provided evidence for the role of cervical sympathetic nerves in thyroid regulation (57). In rats studied 24 weeks after SCGx, an increased goitrogenic response to endogenous or exogenous TSH was found (58). The goiterpromoting effect of chronic SCGx was observed ipsilaterally when a unilateral thyroid lobe denervation was performed. Ipsilateral SCGx amplified the compensatory growth that followed hemithyroidectomy and increased T4 secretion from the remaining lobe (59). An increased mitotic index and a- and p-adrenoceptor binding, concomi-tantly with decreased blood flow, were observed in the thyroid gland of chronic SCGx rats (57-59). Nerve terminals originated in the SCG ganglia also controlled mitotic activity in other tissues. SCG sympathetic stimulation of the eye, for example, resulted in corneal epithelial proliferation and topical applications of NE to eyes that had been deprived of their sympathetic innervation completely reversed the antiproliferative effect of ocular sympathectomy (60). The administration of a-adrenoceptor blockers counteracted the serum T4 reduction found in SCGx rats during the Wallerian degeneration phase (61). Similarly, during the increased autonomic nerve activity obtained by injecting 2-deoxyglucose into mice, the adrenoceptor blocker phentolamine prevented the inhibition of TSH-induced thyroid hormone release (56). A similar cc-adrenergic mediation of NE inhibition of TSH activity on the thyroid was reported *in vitro,* indicating that the NE exerts a direct modulatory effect on thyroid acini (62). a-adrenoceptor blockade, as well as combined a- and p-adrenoceptor blockade, potentiated the inhibition of T4 release observed during Wallerian degeneration of sympathetic nerves (61). This pharmacological study suggests that *(i)* NE acts on inhibitory and stimulatory adrenoceptors of thyroid cells to modify TSH-induced T4 release, and *(ii)* in addition to NE, other inhibitory transmitter(s) are released from degenerating nerve terminals, as indicated by the persistence of depressed T4 levels after the combined administration of a- and p-adrenergic blockers. A number of neuropeptides. such as V1P, NPY. substance P, are present in thyroid nerves and can be considered as candidates for non-

adrenergic effects.

- **Effect of parasympathetic innervation**

Preganglionic parasympathetic input to local thyroid neurons derives from the dorsal motor nucleus of the vagus and is conveyed v/'athe vagal and inferior laryngeal nerves (ILN) and the thyroid nerve (TN) (Fig. 1). Cholinergic fibers enter the thyroid and innervate both the blood vessels and the follicular cells (55). Therefore, the existence of a cholinergic control of thyroid activity has long been envisioned (53). Several studies have indicated that cholinergic agonists impair both basal and TSH-induced thyroid hormone release, suggesting an inhibitory influence of thyroid parasympathetic nerves (54). However, the electrical stimulation of vagal nerves increased ^{131}I labeled hormone release in dogs (63), while vagotomized pigeons, although exhibiting morphological signs of increased thyroid follicle activity, showed reduction of T4 release (64). It should be noted that either vagus nerve section or stimulation probably triggers a number of reflexes ascribed to the whole vagus territory, making it difficult to attribute the results exclusively to local thyroid effects.

We examined the role of thyroid parasympathetic innervation in rats subjected to a regional parasympathetic decentralization by ILN section (65). These animals exhibited an impaired goitro-genic responses and, after hypophysectomy, showed a further involution of the atrophic thyroid gland. ILN section partially impaired thyroid compensatory growth and the increase in thyroid mitotic index found in hemithyroidectomized rats. The findings indicate that parasympathetic nerves are needed to maintain an appropriate thyroid growth in the presence of high circulating TSH levels, as well as a minimal thyroid trophism in the absence of TSH. Whether or not the consequence of ILN section is only vasomotor, or implies a direct effect on acinar function is unknown. Additionally, the extent of involvement of local peptidergic neurons is presently unknown.

AUTONOMIC INNERVATION OF THYROID C-CELLS

- **Effect of sympathetic innervation**

Sympathetic nerve fibers can be traced to the vicinity of thyroid parafollicular C-cells and pharmacological studies have suggested a modulator/ role of circulating catecholamines on calcitonin release by C-cells (55,66). In exteriorized canine thyroid lobes, perfusion of epinephrine was shown to increase calcitonin release, providing that cc-adrenoceptor effects were blocked by administering the a-blocker phentolamine. A number of pharmacological studies in experimental animals and humans support such a p-stimulatory and oc-inhibitory effect of catecholamines on calcitonin release (66). Both a decrease in maximal calcitonin release and a delay in attaining the maximum were detectable in SCGx rats injected with calcium chloride (67). After increasing the hypercalcemic challenge this inhibition was overcome. Blockade of a-adrenoceptors with phenoxybenzamine suppressed the inhibition of C-cell response during anterograde degeneration after SCGx. oc-adrenoceptor blockade by propranolol did not modify calcitonin release during nerve degeneration, but was effective in blunting phenoxybenzamine activity (67). A reasonable explanation for these results is that the supraliminal release of NE, a catecholamine displaying a greater activity on a- than on P-adrenoceptors, from the degenerating terminals produces activation of both types of adrenoceptor on C-cells, the cc-effects being predominant. In a series of studies designed to examine the C-cell response to stress, as well as the participation of SCO neurons in rats injected with turpentine oil, we found that removal of the SCG had a relatively minor effect on stress-induced depression of calcitonin release (68-70).

- **Effect of parasympathetic innervation**

Parasympathetic nerve fibers can be traced to the vicinity of thyroid parafollicular C-cells (55,71,72) although pharmacological studies on the cholinergic modulation of calcitonin secretion are controversial. Surgical section of TN or ILN nerves (73) caused a significant fall in total serum calcium 10 days later, accompanied by a significant decrease of calcitonin levels after ILN section only. Following calcium chloride injection, the increase of serum calcitonin was greater, and the increase of serum calcium levels was smaller, in TN-sectioned rats as compared to sham-operated controls. In ILN-sectioned rats, the secretory response of calcitonin and the hypercalcemia achieved after a calcium chloride challenge was significantly greater than in controls. A correlation study of the data indicated that the slopes of correlation between serum calcium and calcitonin levels in TN- and ILN-sectioned rats were significantly greater than in controls. The results were interpreted to indicate that ILN and TN have an inhibitory parasympathetic effect on C-cell secretion (73).

AUTONOMIC IMMERVATIOM OF PARATHYROID GLANDS

- **Effects of sympathetic innervation**

Early studies provided histochemical evidence on the autonomic innervation of parathyroid cells. Using the horseradish peroxidase-retrograde transport method after injection into the parathyroid gland, numerous labeled cells were observed in the caudal half of ipsilateral SCG while in the medulla oblongata, labeled neurons were found in the dorsal nucleus of the vagus (72) (Fig. 1). *In vitro,* adrenoceptor agonists affected PTH release in parathyroid cells and slices. In intact rats and cattle pharmacological stimulation of p-adrenoceptors augments PTH secretion, while cc-adrenoceptor activation is fol-

lowed by a decrease in PTH (66). Furthermore, hypocalcemia and/or increases of circulating PTH levels are common findings during stress. The response of parathyroid glands of rats whose SCO nerves underwent Wallerian degeneration was tested by injecting the hypocalcemic agent EDTA (74). Shortly after SCGx, a significant impairment of parathyroid cell's response to hypocalcemia was observed. As in the case of calci-tonin or T4 release, the major inhibitory effect of NE released during nerve degeneration was mediated by a-adrenoceptors. We also examined the effect of chronic SCO ablation on PTH and calcium levels in rats. By two weeks after SCGx, basal levels of PTH were increased in the presence of unchanged calcium levels, as compared to sham-operated rats. The general pattern of PTH response differed significantly showing a slower response in SCGx rats the results indicating a modified sensitivity of parathyroid cells after chronic regional sympathetic denervation. To assess whether the cervical sympathetic nervous system was involved in conveying information to parathyroid cells on stress, a bilateral SCGx was performed in rats 14 days before to turpentine oil administration (68). SCGx blocked partially the PTH increase observed after turpentine oil injection. These results indicate that the stress resulting from turpentine oil administration affects PTH homeostasis and suggest the partial involvement of the sympathetic neural pathway in conveying information on stress to the parathyroid glands. In a clinical follow-up of this study, we reported the correlation of serum and urinary calcium levels with the degree of psychopathology in stressed patients (75). A non-parametric bivariate correlation analysis revealed a negative correlation of psychopathology with serum calcium, while urinary calcium levels correlated positively. In view of the modu-latory role of the peripheral autonomic innervation on thyroid C-cells and parathyroid function above documented, it was tested the potentiality of PTH and calcitonin to modify autonomic ganglion activity. In our studies, cholinergic neurotrans-mission in the rat SCG was modulated by PTH and calcitonin, presumably as an indication of the feedback loop linking sympathetic activity with thyroid and parathyroid glands (76,77). This kind of regulating system has previously been described for T4 effects on SCG function (78).

- **Effect of parasympathetic innervation**

Pharmacological data support the existence of cholinergic receptors in parathyroid cells (66). Cholinergic agonists decreased PTH release from bovine parathyroid slices, an effect that was antagonized by atropine. The systemic administration of cholinergic agonists to rats decreased serum PTH levels, the effect being also blocked by atropine. The administration of atropine alone augmented serum PTH levels, suggesting that a musca-rinic cholinergic tone with an inhibitory effect on PTH release is present *in vivo* (66). We examined the effects of regional surgical parasympathetic decentralization of parathyroid glands on PTH secretion in rats. Specifically, we wanted to assess the physiological consequences of the surgical section of TN and ILN on basal PTH secretion as well as on the response of PTH to an appropriate hypocalcemic challenge (73). Both surgical sections significantly decreased parathyroid neuronal 3H-choline uptake (an index of cholinergic innervation). A significant fall in total serum calcium and a significant increase in serum PTH were observed 10 days after TN and ILN sections. When parathyroid sensitivity to a hypocalcemic challenge was assessed, a significantly greater serum PTH response to EDTA was detectable in TN- or ILN-sectioned rats. A correlation study of the data indicated that the slopes of correlation between serum calcium and PTH levels in TN- and ILN-sectioned rats were significantly steeper than in controls. The results indicate that ILN and TN exert an inhibitory parasympathetic influence on parathyroid secretion (73). In summary, the results about calcitonin and PTH release in rats during Wallerian degeneration after SCGx, turpentine oil-induced stress, or during the chronic absence of sympathetic or parasympathetic neural input, support the view that peripheral autonomic nerves play a modulatory role of endocrine cell responsiveness to the appropriate hyper or hypocalcemic stimulus. This modulatory level is superimposed upon the feedback level of organization of calcium homeostasis (13).

CIRCADIAN ORGANIZATION OF THE IMMUNE RESPONSE

- In the last 15 years, the concept that in almost every physiologic circumstance the central nervous system (CNS) and the immune system interact has emerged, giving rise to neuroimmunology as an independent discipline. One conse quence of CNS regulation of immune response is the strong circadian organization that immune function exhibits, driven by signals derived the nervous structures linked to the circa dian apparatus (79).

Two immune circadian systems have been identified: *(i)* the circulation of T, B, or natural killer (NK) lymphocyte subsets in peripheral blood, and *(ii)* the density of epitope molecules (e.g. CD4, CDS) at their surface, which may relate to cell reactivity to antigen exposure. In CB A mice, for example, macroph-age spreading and ingestion ability are significantly lower at the beginning and higher at the end of the dark period, while a significant increase in blood T lymphocytes and helper/inducer T lymphocyte percentages occurred during the dark period
(80). Studies in mice placed on 12-12 hr light-dark schedule showed that hours after lights on had a significant effect on body temperature, percentage of splenic B cells, T pan, T helper and T suppressor cells, and absolute numbers of T pan cells
(81). In addition, the response of splenocytes or lymph node cells to immunosuppression strongly depended upon circadian time of exposure (82). Changes in lymphocyte subset populations can be attributed to time of day-associated changes in

cell proliferation in immunocompetent organs as well as to nycto-hemeral modifications in lymphocyte release and traffic among lymphoid organs.

During the last years we examined the regulation of levels and circadian rhythinicity of ornithine decarboxylase (ODC) activity in rat submaxillary lymph nodes and spleen during the immune reaction, as an indicator of the extent of cell proliferation in lymphoid tissue. In both immunized and nonimmunized rats, we reported a significant diurnal variation of submaxillary lymph node ODC displaying maximal activity at 13:00-17:00 hratthe same time of maximal lipopolysaccharide- and concanavalin A-induced cell proliferation in submaxillary lymph nodes. We also reported in rat spleen daily rhythms in NK. activity and lipopoly-saccharide-induced cell proliferation exhibiting a maximum at midnight and at early morning, respectively, while concanavalin A-induced lymphocyte proliferation peaked at midday (83). Splenic ODC showed a maximum at morning hours, which coincided temporally with changes in some immune-related parameters, e.g. mitogenic activity.

Theoretically, two possible pathways through which the CNS may modulate the circadian organization of the immune response should be considered. One is purely neuroendocrine and involves the strong circadian profile of secretion of many hormones, like corticoids or melatonin. Another includes the direct circadian control of immune reactivity through the autonomic nervous system innervating the lymphoid organs (3). Their relative importance is discussed below.

- **Role of autonomic innervation in circadian control of lymphoid organs**

Lymph nodes in the cervical region, like the submaxillary lymph nodes, receive sympathetic innervation from neurons located in the SCG, whose activity shows a strong circadian functional organization. Based on this, an experimental model was developed comprising the submaxillary lymph nodes and the local ipsilateral manipulation of regional sympathetic nerves and/or the ipsilateral manipulation of the regional parasympathetic nerves (conveyed through the lingual nerve chorda tympani) (Fig. 2). This experimental approach allowed us to uncover the local effects of the autonomic nerves in the ipsilateral dener-vated lymph nodes, when compared with the contralateral, sham-operated side. In this way, any difference in the immune response between ipsilateral and contralateral lymph nodes could be ascribed to a local effect of the nerves, independently of the occurrence of systemic effects of the surgical manipulation itself.

It is generally accepted that SCG neurons increase their activity at night, e.g. nicotinic cholinergic transmission in rat SCG, which is instrumental for stimulation of ganglion neurons and is triggered by acetylcholine release from preganglionic fibers, exhibited a circadian organization with a high activation during the first half of the night (2). Hence it was hypothesized that the nyctohemeral changes in submaxillary lymph node ODC activity could be related to a circadian signal conveyed *via* the sympathetic neurons arriving at the lymph nodes (83-86). Diurnal rhythm of cell proliferation after Freund's adjuvant injection, as assessed by ODC, was measured in submaxillary lymph nodes of rats subjected to an ipsilateral SCGx and/or a chorda tympani section 10 days earlier. In sympathetically denervated plus parasympathetically decentralized submaxillary lymph nodes, the normal diurnal variation, that is, a maximum in cell proliferation at 17:00 hr, became significantly suppressed. It was thus concluded that an important regulation of circadian rhythms in cell proliferation in lymph nodes derived from the autonomic nerves.

- **Role of melatonin in circadian control of lymphoid *c-* organs**

A compelling information has accumulated that biological time measurement in adult and fetal mammals is critically dependent upon the melatonin signal released by the pineal gland (84,87-89). The mechanisms sensitive to melatonin, which mediates the clock message, may reside in the brain, in the biological clock itself, i.e. the suprachiasmatic nuclei. There are also data indicating that melatonin receptors are universally distributed throughout in the body and can convey circadian-meaningful information to every cell in the organism, playing the role of an "internal synchronizer". Among melatonin's versatile functions, immunomodulation has emerged as a major effect. Indeed the melatonin rhythm seems to be a very important efferent pathway of the suprachiasmatic nuclei to impose synchrony to the immune system. The brain, as well as the peripheral immune cells, can be thus able to encode an accumulated memory of melatonin signals, thereby defining time intervals at the 24-hour cycle as well as the annual scale. Pinealectomy (Px) or any other experimental procedure that inhibits melatonin synthesis and secretion induces in general a state of immunodepression that is counteracted by melatonin in several species (79). *In vivo,* melatonin displays an immunoenhancing effect, particularly apparent in immunodepressive states on various immune parameters. Melatonin administration augments antibody responses, such as plateforming cell activity, cytotoxic T-cell response against vaccinia virus and antibody dependent cellular cytotoxicity, corrects immunodeficiency that followed stress, immunosuppressant drugs or viral infections, and prevents apoptosis in hematopoietic cells and thymocytes (89).

We examined the effect of pineal suppression and of melatonin replacement on diurnal variations in submaxillary lymph node ODC activity in rats subjected to Px, bilateral SCGx or their respective sham-operations 12 days earlier and treated with

Freund's complete adjuvant (85). From the day after surgery, rats received 11 daily injections of 30 lag of melatonin or vehicle, one hour before lights off. In Px rats, ODC activity decreased by about half, while it still exhibited significant diurnal variations with maxima at 13:00 hr. After a bilateral SCGx, abolition of circadian rhythmicity in ODC and depression of enzyme activity to about 1/2 - 1/3 of controls were found in submaxillary lymph nodes. Administration of melatonin at late evening restored ODC levels and amplitude of diurnal rhythmicity both in Px and SCGx rats. Likewise, melatonin treatment was effective to augment enzyme levels and amplitude of circadian variations in submaxillary lymph node ODC activity of sham-operated controls.

In the spleen of immunized and nonimmunized rats, significant diurnal variations in ODC activity were detectable, with a maximum at early morning, acrophases after Cosinor analysis varying from 08:45 to 11:00 h (86). In Px or SCGx, immunized rats, splenic ODC activity attained values significantly lower than those of immunized sham-operated controls, while amplitude decreased significantly by about one-third. Administration of melatonin (30 jag/animal for 11 days) significantly augmented mesor levels of splenic ODC activity and increased the amplitude of the its rhythm both in Px and in SCGx rats. The results are compatible with the view that the pineal gland plays a role in circadian changes of immune responsiveness in rat submaxillary nodes and spleen *via* an immunopotentiating effect of melatonin on lymph node and splenic cell proliferation.

Another subject addressed in our experiments concerned as to whether melatonin affected the diurnal change in activity of local autonomic nerves in submaxillary lymph nodes and spleen, which are concomitant with the immune response. Indeed, the interaction between the CNS and the immune system is a bidirectional process, immunocompetent cells affecting local neural processes by paracrine means through the release of cyto-kines and by endocrine means on the CNS *via* the bloodstream (90). During the immune reaction there was an increase of sympathetic and parasympathetic activity in local nerves, as indicated by the significant increase of tyrosine hydroxylase (TH) activity of submaxillary lymph nodes and spleen, and of 3H-choline conversion into acetylcholine and of norepinephrine and choline uptake in submaxillary lymph nodes of rat administered with Freund's adjuvant (85,86). Submaxillary lymph node

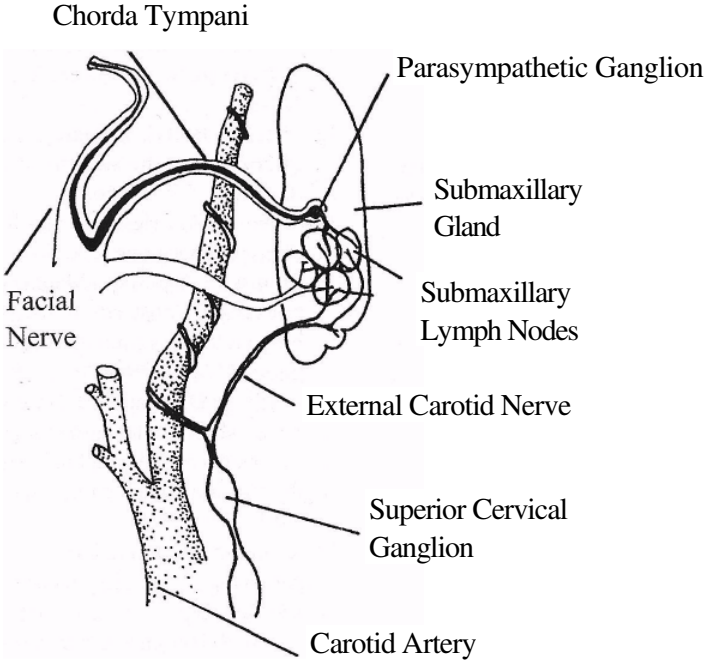

***Figure** 2. Sympathetic and parasympathetic innervation of submaxillary lymph nodes. From Ref 3.*

and splenic sympathetic activity attained their maximum at early night, while cholinergic activity in lymph nodes peaked during the afternoon. Px evoked a significant decrease of submaxillary lymph node TH and acetylcholine synthesis. Each effect of Px was significantly counteracted by melatonin injection. In sham-operated rats, melatonin also augmented lymph node TH activity and acetylcholine synthesis. In the same series of experiments, immunization increased significantly mesor values of splenic TH activity, whereas neither Px nor SCGx affected circadian rhythm parameters. Melatonin treatment augmented mesor values of splenic TH rhythm and increased its amplitude in Px, SCGx or sham-operated rats. Therefore, melatonin may act in part by modifying circadian rhythmicity of neural signals conveyed to the immunocompetent organs *via* the autonomic nerves.

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