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Regulation of noradrenergic neuronal activity in the rat locus coeruleus by serotoninergic afferents

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From an anatomical point of view, the serotonin axonal varicosities in the locus coeruleus have been defined as nonsynaptic terminals originating from the raphe dorsalis, raphe centralis, and raphe pontis. Electrolytic or chemical destruction of these serotoninergic afferents has produced pronounced increases in the activity of the noradrenaline biosynthetic enzyme, tyrosine hydroxylase, in the locus coeruleus and in the noradrenaline metabolite DOPEG in the terminal field of the hippocampus and cerebral cortex. A good correlation has been observed between the depletion of serotonin and the increase in tyrosine hydroxylase activity in the locus coeruleus after chemical denervation of serotoninergic terminals. Electrical stimulation of serotonin cell bodies has increased noradrenaline levels in the locus coeruleus and cerebral cortex. Altogether these data suggest that the function of the ascending noradrenergic system originating from the locus coeruleus might be largely dependent on its interaction with serotonin-containing neurons in the raphe system.

In human brain specimen, a bluish color due to melanin pigments, denotes a region of the pontic tegmentum known as the locus coeruleus (LC) (Reil, 1809, cited in Ziehen, 1920). Over a century after Reil, Riley (1943) described five pigmented nuclei in this region: the nucleus LC, nucleus accessorius LC, nucleus nublis, nucleus pigmentosus tegmento-cerebelloxis, and the nucleus pigmentosus tegmento-pontinus.

It is now well established that the LC is a small pontine nucleus which contains more than 40% of all the noradrenaline (NA) in the rat brain (Dahlström & Fuxe, 1964; Fuxe, Goldstein, Hökfelt, & Joh, 1970; Olson & Fuxe, 1971; Pickel, Joh, & Reis, 1977; Shimizu & Imamoto, 1970; Swanson & Hartman, 1975). Efferents emanating from the LC reach all the major brain areas of the central nervous system, and therefore it is not surprising that the LC has been implicated in such diverse functions as respiration, motivation, micturition, and sleep. Just as the LC sends projections throughout the neuraxis, it also receives a complex heterogeneous network of afferents (Belin et al., 1979; Cheney, Lefevre, & Racagni, 1975; Leger & Descarries, 1978; Pickel et al., 1977; Simon, Le Moal, & Calas, 1979). These major inputs, especially the serotoninergic raphe afferents, may be of particular importance in relation to how the LC coordinates its multiple activities. For instance, it has been suggested that serotonin(5-HT)-containing neurons of the raphe system and NA cells from the LC have mutually antagonistic regulatory effects upon sleep or waking (Jouvet, 1972; Pujol, 1972). The aim of this report is to present an anatomical and biochemical overview of the hypothesis that 5-HT neurons in the raphe system are involved in the regulation of NA neuronal activity in the LC.

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ANATOMICAL EVIDENCE FOR A SEROTONINERGIC INNERVATION OF THE LC

The LC contains a considerable amount of 5-HT (Palkovits, Brownstein, & Saavedra, 1974) and its specific enzyme tryptophan hydroxylase (Renson, 1973; Brownstein, Palkovits, Saavedra, & Kizer, 1975; Saavedra & Axelrod, 1975). Immunocytochemical (Pickel et al., 1977) and autoradiographic (Leger & Descarries, 1978) investigations have confirmed that the LC receives a dense serotoninergic innervation. These studies have revealed

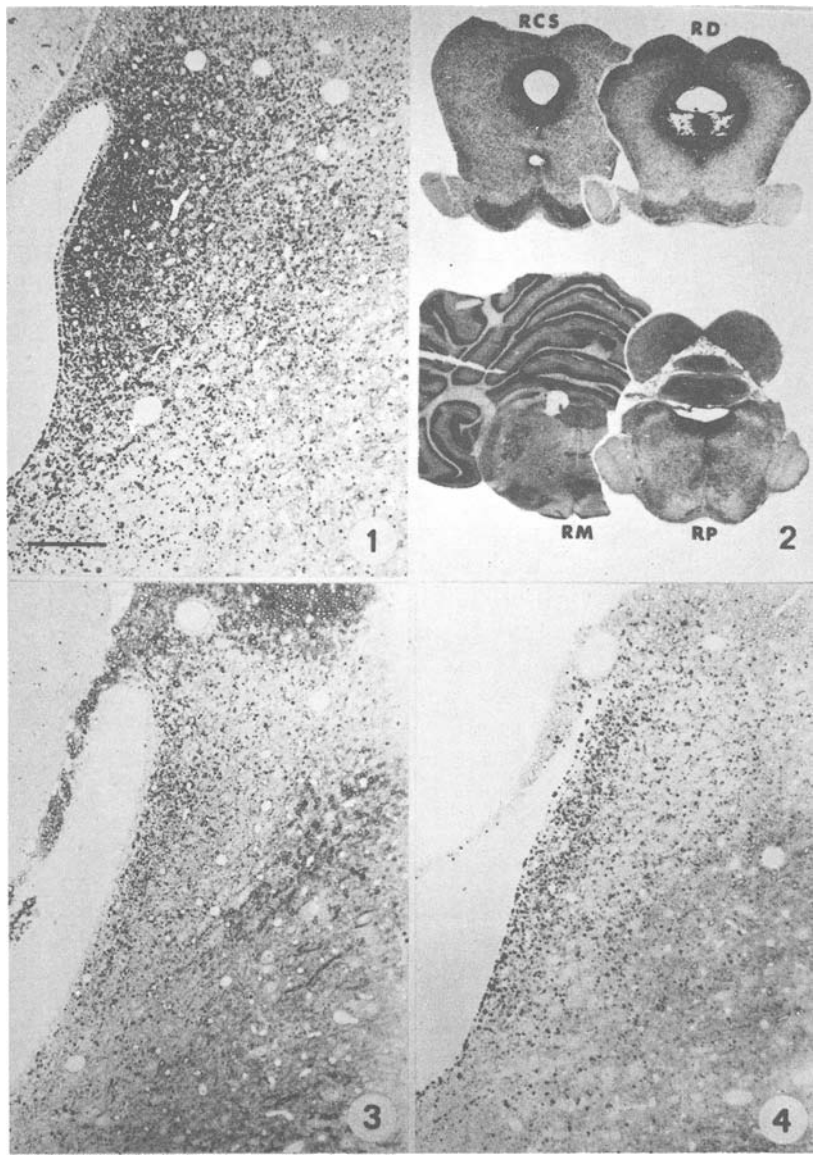

Figure 1. Anatomical evidence for a serotonergic innervation of the LC in the rat (from Leger et al., 1980). (1) Autoradiograph of the LC region in a nonlesioned rat after an intraventricular injection of ^{3}H-5-HT (10^{-4}M). The accumulation of numerous silver grains dispersed between the cell bodies of the LC corresponds to the accumulation of ^{3}H-5-HT in serotonergic axonal varicosities. (2) Frontal sections showing typical electrolytic lesions of the raphe dorsalis (RD), raphe centralis (RC), raphe pontis (RP), and raphe magnus (RM). Electrolytic lesions were performed as described in the Figure 2 caption. (3) Autoradiograph of the LC region 6 weeks following an electrolytic lesion of the RD. There is a noticeable decrease in the number of reactive axonal varicosities when compared with those of the control rat. The supra-ependymal border disappeared after a lesion of the RD or RC. (4) Autoradiograph of the LC region 4 weeks after an electrolytic lesion of the RP. The decrease in the number of reactive terminals is less pronounced than that seen after RD or RC lesion. The supra-ependymal border is still labeled after a RP lesion.

that the axonal varicosities appear to be evenly distributed throughout the LC. Interestingly, electron microscopic observations have demonstrated that only a small proportion (10%) of the reactive terminals display morphologically defined synapses and that for the most part these contacts are axodendritic.

Further anatomical and biochemical approaches have demonstrated that the serotoninergic innervation of the LC originates in three raphe nuclei, 40% from the raphe dorsalis (RD), 24% from the raphe centralis (RC), and 21% from the raphe pontis (RP) (Leger, McRae-Degueurce, & Pujol, 1980; Morgane & Jacobs, 1979). Both of these investigations showed that the raphe magnus (RM) does not make a contribution to the 5-HT innervation of the LC (Figure 1). The investigation by Morgane and Jacobs (1979) determined, to a certain degree, the distribution of the fibers from the various raphe nuclei in the LC. For instance, the RP appeared to send projections more to the ventrolateral portion of the LC than to the dorsomedial portion. Projections from the RD and RC appeared to be located more in the rostral and medial parts of the nucleus than in its caudal limits. The projections

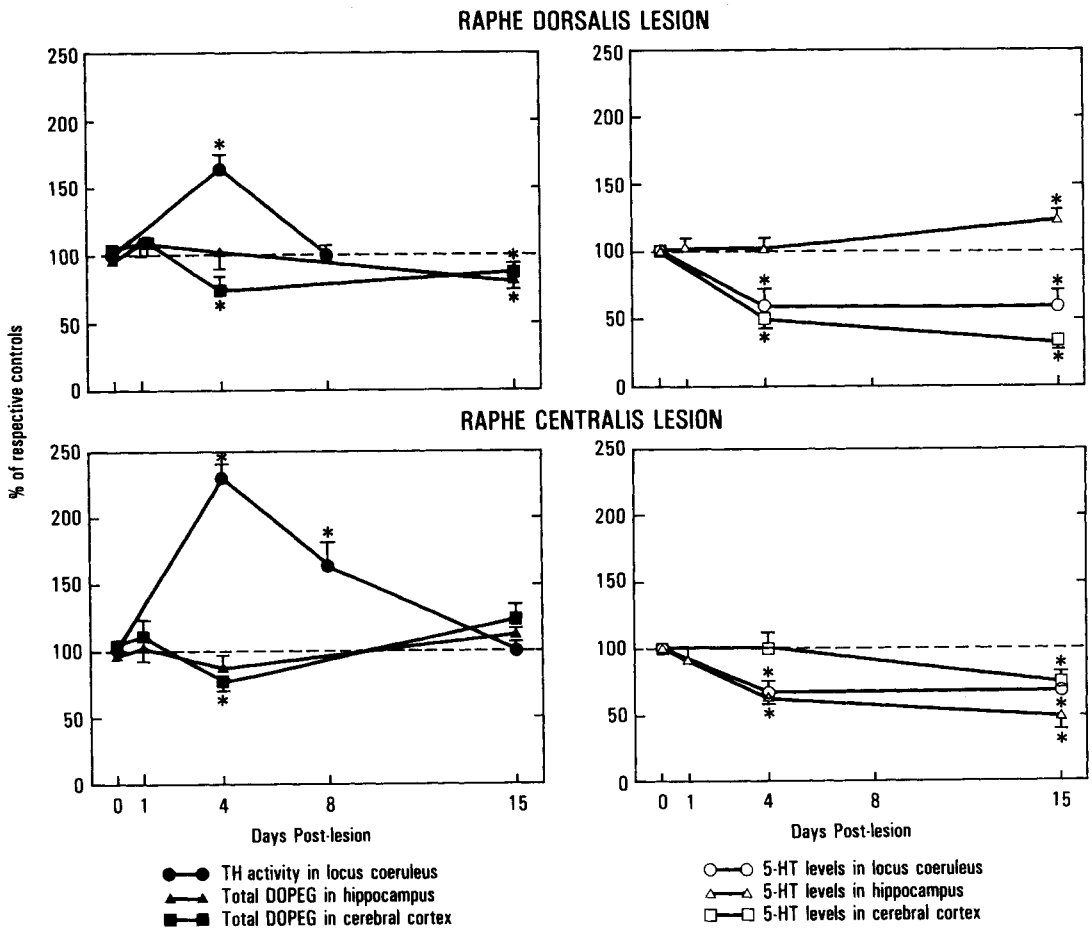

Figure 2. Effect of an electrolytic lesion of the raphe dorsalis and raphe centralis on NA metabolism and 5-HT levels in the LC and in noradrenergic projection fields in the rat. The upper panel (left) depicts the time course of variations of TH activity in the LC, DOPEG in the hippocampus, and DOPEG in the cerebral cortex 1, 4, and 15 days after an electrolytic lesion of the RD. On the right are shown the decreases in 5-HT measured simultaneously in the tissue samples after the lesion. The lower panel (left) depicts the time course of variations in the same biochemical parameters 1, 4, and 15 days after an electrolytic lesion of the RC. On the right are shown the decreases in 5-HT content in the corresponding tissue samples. Electrolytic lesion of the raphe nuclei were performed under chloral hydrate (400 mg/kg, ip) anesthesia, with a nichrome wire coaxial insulated electrode (0.5-mm diameter) set at an angle of 45° from the vertical axis. To lesion the RD, the electrode was introduced successively at coordinates AP 5, DV 5, L 0, and AP 6, DV 4.9, L 0 (atlas of König & Klippel, 1974) and a current of 3 mA was applied for 4 sec. To destroy the RD, a current of 3 mA was applied for 3 sec to an electrode located successively at the following coordinates: AP 3.4, DV 1.9, L 0, and AP 3.4, DV 2, L 0. TH activity was assayed according to Nagatsu, Levitt, and Udenfriend (1964). DOPEG levels were measured according to the radioenzymatic procedure of Dennis and Scatton (1982). 5-HT levels were determined by high-pressure liquid chromatography with electrochemical detection (Semerdjian-Rouquier, Bossi, & Scatton, 1981). Results are the means with SEM of data obtained on 8-20 rats per groups. *$p < .05$ versus respective controls.

from the RD were always denser than those arising from the RC and RP, which agrees with the findings of Leger et al. (1980).

The LC not only receives 5-HT afferents from the raphe nuclei but also contains 5-HT perikarya (Steinbusch, 1981). These cells are smaller than the noradrenergic cells present in that same nucleus.

These observations, taken together, provide the anatomical substrate for suggesting an interaction between 5-HT terminals and NA-containing cells in the LC. However, the relative paucity of synaptic contacts formed by the 5-HT varicosities raises a question concerning a neuromodulator or neurotransmitter role for 5-HT liberated from these terminals in relation to regulating NA neuronal activity in the LC. In the following sections, the possible mechanisms whereby 5-HT interacts with NA neuronal activity in the LC will be discussed.

BIOCHEMICAL EVIDENCE FOR A SEROTONINERGIC CONTROL OF NA NEURONS IN THE LC

Lesion of 5-HT Containing Cell Bodies

After the establishment of the origin of the 5-HT innervation of the rat LC, investigators' interest turned to investigating whether these projections took part in the control of NA neuronal activity in this nucleus. To this end, specific electrolytic lesions of the individual raphe nuclei were performed and time course variations in the activity of the catecholamine biosynthetic rate-limiting enzyme tyrosine hydroxylase (TH) and endogenous 5-HT content were measured in the rat LC. Moreover, the NA deaminated metabolite 3,4-dihydroxyphenylethyleneglycol (DOPEG) and 5-HT content were determined in some LC terminal fields (e.g., hippocampus and cerebral cortex).

As indicated in Figure 2, lesions of individual raphe nuclei (RD and RC) provoked significant increases in TH activity in the LC (McRae-Degueurce et al., 1982). The increase in TH activity progressed until it reached its apex on the 4th day; TH activity returned to normal levels between 8-15 days postlesion. It should be mentioned that lesions of the RP also provoked significant increases in TH activity in LC, whereas lesions of the RM (a 5-HT nucleus which does not innervate the LC) failed to modify TH activity in the LC (McRae-Degueurce et al., 1982). Immunotitrations of TH in the LC showed that inactivation of the 5-HT system produced a significant increase in the number of enzyme molecules in the LC (McRae-Degueurce et al., 1982), suggesting an induction of TH. This indicates that the RC and RD exert an inhibitory influence on the transsynaptic induction of TH in the LC.

In contrast to the changes in TH activity in the LC, the total DOPEG levels in the noradrenergic terminal fields of the hippocampus and cerebral cortex were slightly decreased following individual lesions of the raphe nuclei (Figure 2). The significant decreases in the endogenous 5-HT content in the LC, hippocampus, and cerebral cortex attested to the efficacy of the raphe lesions (Figure 2).

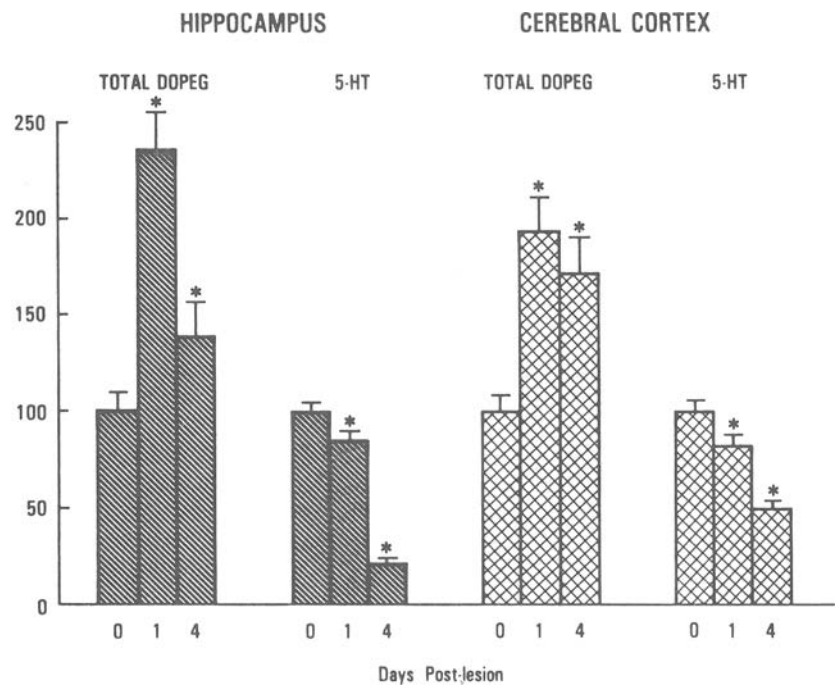

Figure 3. Effect of combined lesions of the raphe dorsalis and raphe centralis on total DOPEG and 5-HT levels in the hippocampus and cerebral cortex in the rat. The details of the biochemical techniques are given in the legend of Figure 2. Results are the means with SEM of data obtained on 8 rats per group. *p < .05 versus respective controls.

Since the individual RD or RC lesions only slightly modified the total DOPEG levels in the hippocampus or cortex, the effect of lesions of both raphe nuclei on this biochemical parameter was investigated. As shown in Figure 3, total DOPEG levels were markedly enhanced at 1 and 4 days postlesion in both structures. In the hippocampus, the effect observed at 4 days was less in magnitude than that seen at 1 day. It should also be noted that the 5-HT content was drastically reduced at 4 days relative to 1 day postlesion in both structures (Figure 3).

Lesions of 5-HT Terminals in the LC

In view of the heterogeneous cell body population in the raphe nuclei (Belin et al., 1979; Cheney et al., 1975; Ochi & Shimizu, 1978; Uhl, Goodman, & Snyder, 1979), it appeared necessary to investigate whether specific destruction of 5-HT terminals with the neurotoxin 5,6-dihydroxytryptamine (5,6-DHT) produced metabolic changes in TH activity in the rat LC similar to those observed after lesion of the 5-HT cell bodies. This hypothesis was further investigated by simultaneously measuring 5-HT levels and TH activity in the LC when the extent of destruction of 5-HT terminals was varied by increasing doses of the neurotoxin (McRae-Degueurce & Pujol, 1979) (Figure 4). A close correlation between the two biochemical parameters was observed for three doses of 5,6-DHT. The 50-μg dose caused a greater decrease in the 5-HT content than the 25- or 15-μg doses, and TH activity increased as a function of the depletion of 5-HT. Using immunotitration, it was found that the 50-μg dose induced a significant increase in enzyme concentration (McRae-Degueurce et al., 1982).

A final observation underscores the specificity of both of the phenomena described above (Figure 5). Intraventricular injections of 5,6-DHT caused a drastic reduction in the 5-HT levels in the LC for 15 days (24% to 30% of the controls), but by 4 months the 5-HT content had returned to almost normal control levels. When a lesion of the RC was performed in rats that had received 5,6-DHT 15 days prior to lesion, there was only a minor increase in TH activity in the LC. However, a lesion of the RC performed 4 months after the neurotoxin provoked once again the characteristic increase in TH activity in the LC. Both of these results emphasize that the presence of 5-HT terminals in the LC is essential for the TH activity response to inactivation of the 5-HT system (McRae-Degueurce, Leger, Wiklund, & Pujol, 1981). Moreover, they suggest that the 5-HT neurons have the capacity to regenerate and resume normal regulation in the LC.

Stimulation of the Raphe Nuclei

The evolving hypothesis that NA neuronal activity in the LC is regulated by 5-HT afferents was examined in the above paragraphs as the response of TH activity in

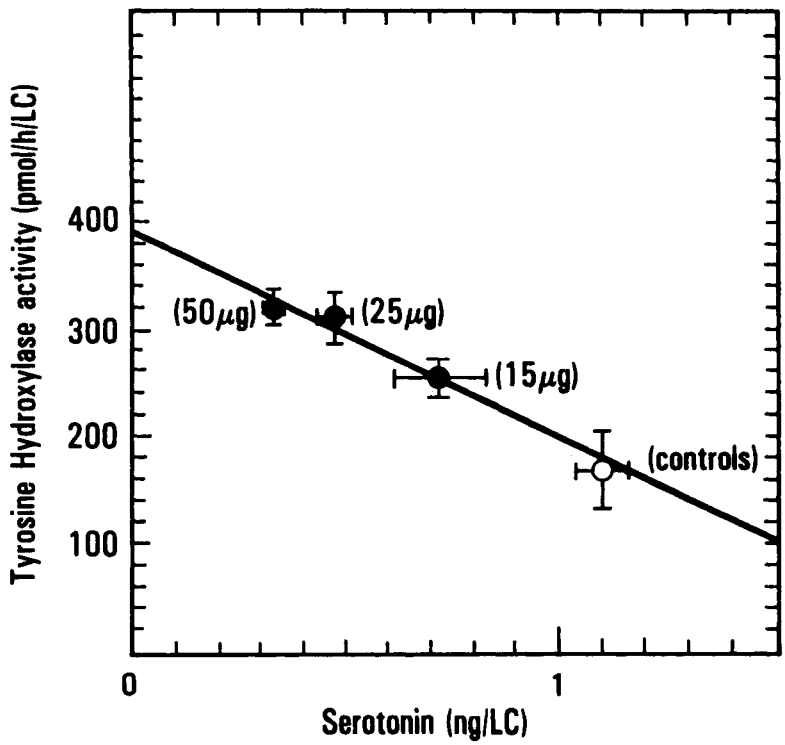

Figure 4. Correlation between the decreases in 5-HT levels and increases in TH activity in the rat LC 4 days after intraventricular injection of three doses of 5,6-DHT. The numbers in parentheses indicate the dose of 5,6-DHT injected. The TH activity is expressed as pmoles dopa/LC/h and the 5-HT contents in ng/LC. The correlation was significant at $p < .001$ for the two parameters.

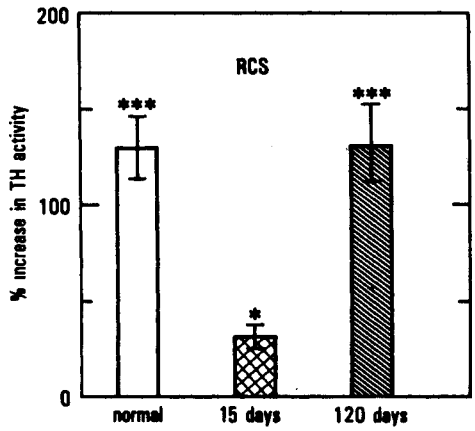

Figure 5. Increases in tyrosine hydroxylase activity in the LC provoked by lesion of the raphe centralis at different time periods after 5,6-DHT-induced axotomy. Electrolytic lesion of the raphe centralis was achieved 15 days or 120 days after intraventricular injection of 5,6-DHT, and TH activity was measured in the LC 4 days after the RC lesion. On the left, the change in TH activity in vehicle-injected rats (+120%, $p < .001$). In the middle, the change in TH activity in rats receiving 5,6-DHT (75 μg) 15 days beforehand (+30%, $p < .05$). On the right, the change in TH activity in rats pretreated with 5,6-DHT (75 μg) 120 days prior to RC lesion (+120%, $p < .001$). The results are expressed as a percentage of the respective controls. Vertical bars indicate the SEM. *$p < .05$. ***$p < .001$.

the LC to inactivation of the 5-HT system. It was, therefore, of interest to investigate the changes in NA levels (an index of TH activity, since this enzyme is the rate-limiting step in the synthesis of NA) after an electrical stimulation of the RC and RD nuclei. This experiment was performed with freely moving rats. The time course of variations in the NA content in the LC was measured both in rats with intact 5-HT terminals and in rats with degenerated 5-HT terminals. As depicted in Figure 6, a substantial increase in the NA content in the LC was observed at both 2 and 4 days following the electrical stimulation of the RC. This was not the case in rats with degenerated 5-HT terminals. There was a notable accumulation of 5-HIAA in the LC at 30 min, 2 days, and 4 days after the stimulation; this was abolished in rats with degenerated 5-HT terminals (not shown). It should also be mentioned that 30 min after the stimulation free DOPEG levels in the cortex, but not the hippocampus, were significantly elevated (in preparation). Electrical stimulation of the RC also caused characteristic behavioral alterations (repetitive head movements, gnawing, bruxism, nystagmus, vibrissal movements) and a slight elevation (20 mm Hg) of mean blood pressure.

DISCUSSION AND CONCLUSIONS

Globally, all these results strongly suggest that 5-HT is involved in the transsynaptic regulation of NA neuronal activity in the LC. This is compatible with the anatomical data that have defined the origin and morphology of the 5-HT terminals in the LC (Leger & Descarries, 1978; Leger et al., 1980; Morgane & Jacobs, 1979; Pickel et al., 1977). The control of NA cells in the LC by 5-HT fibers is of particular interest, since, for the most part, there are few synaptic contacts on NA cells, suggesting that 5-HT is acting as a neuromodulator.

In view of the present biochemical data, it is not yet clear whether the control exerted by serotonergic afferents over LC NA cells is inhibitory or facilitatory in nature. In fact, our results are divided concerning the actual role of 5-HT. The increase in TH activity in the LC observed after electrolytic lesions of RD or RC tends to provide evidence that 5-HT exerts an inhibitory control on NA neuronal activity in the LC. The close correlation observed between the extent of 5,6-DHT-induced denervation of 5-HT neurons and the increase of TH in the LC indicates a causal relationship between these two biochemical events. Both investigations in which the 5-HT system was deafferented showed that there is an increase in TH activity which reaches its apex at 4 days and then returns to normal levels by Day 15. Further analysis of these increases indicated that the elevation of TH activity in the LC corresponds to an increase in the number of

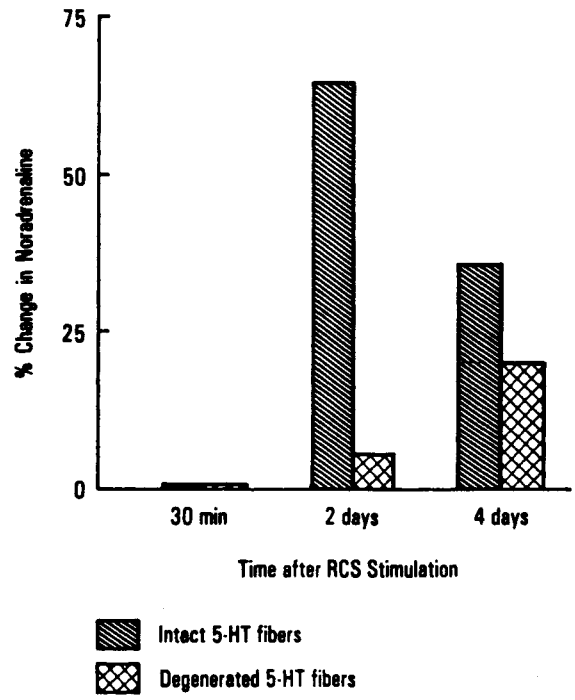

Figure 6. Changes in noradrenaline content in the LC 30 min, 2 days, and 4 days after an electrical stimulation of the raphe centralis in the freely moving rat. Biphasic rectangular pulses at a frequency of 10 Hz (2 msec duration, 6 V) were delivered for 30 min via a bipolar concentric electrode (implanted stereotaxically in the raphe centralis 2 days before the experiment) to rats with an intact 5-HT innervation or to rats that had received 5,7-DHT 15 days previously. 5,7-DHT (200 μg/10 μl) was injected intracerebroventricularly to rats pretreated with desipramine (25 mg/kg, ip) and nomifensine (10 mg/kg, ip) to prevent destruction of catecholaminergic neurons. Results are the means with SEM of data obtained on 6-8 rats per group and are expressed as percent change versus control values. In all groups, SEM represented less than 5% of absolute basal values.

enzyme molecules. These results, therefore, possibly reflect transneuronal inhibitory control of TH activity in NA neurons by 5-HT fibers. Our results showing substantial increases in hippocampal and cortical total DOPEG levels following the lesions of both RD and RC agree with those of Kostowski et al. (1974), who demonstrated that similar lesions provoked significant elevations in another NA metabolite, 3-methoxy-4-hydroxyphenylethyleneglycol sulphate (MOPEG-SO_4), in these same areas. Preliminary experiments showing that local infusions of the sodium channel blocker tetrodotoxin in the RC or RD produced significant increases in MOPEG and 3,4-dihydroxyphenylacetic acid (DOPAC) levels (indexes of NA neuronal activity, Curet, Dennis, & Scatton, 1985) in the LC (in preparation) also favor an inhibitory role of 5-HT in the LC. We do not have any direct evidence for modification of the firing rate of LC noradrenergic neurons in our experimental conditions, but it is accepted that DOPEG and DOPAC levels are reliable indexes for NA neuronal activity and both of these biochemical parameters were increased in NA cell bodies or terminals after acute interruption of neuronal transmission (tetrodotoxin infusion) between the raphe and the LC or in terminal fields following the double raphe lesions.

Thus, altogether, these data support the view that RD and RC 5-HT neurons exert an inhibitory influence on NA neurons originating in the LC. In contrast, another set of data seems to indicate that 5-HT exerts a facilitatory control over NA metabolism in the LC. Thus, electrical stimulations of the RC provoked significant increases in the NA content of the LC (reflecting an increase in TH activity) and an increase in DOPEG levels in the cerebral cortex. This effect was attenuated when the stimulation was performed in animals previously deprived of their 5-HT fibers, which eliminates a generalized effect of the stimulation on NA metabolism. Moreover, individual lesions of the RD or RC provoked decreases in DOPEG levels in the hippocampus and cerebral cortex. These data would therefore favor the hypothesis that RC 5-HT neurons exert a facilitatory control over LC NA neurons. This conclusion is also supported by other studies showing that 5,7-DHT- or 5,6-DHT-induced destruction of 5-HT terminals produces a decrease in normetanephrine levels (an index of NA synaptic activity) and TH activity in the cerebral cortex of the rat (Racagni, Mocchetti, Calderini, Battistella, & Brunello, 1983; Renaud, Buda, Lewis, & Pujol, 1975). Moreover, electrophysiological studies (Watabe, 1980) have shown that electrical stimulation of the RD causes an excitation followed by a long-lasting inhibition of LC cells. The latter inhibition is thought to be secondarily triggered by activation of axon collaterals of the LC neurons and subsequent self-inhibition of LC cells. Finally, electrical stimulation of the RD dramatically increases local cerebral glucose utilization in the LC (A. Cudennec, personal communication, 1985), which suggests a facilitatory influence of the RD upon functional events in the LC. Thus, from the above data, no clear picture emerges concerning the excitatory or inhibitory nature of the serotonergic control of LC cells, and further experiments are clearly needed in order to solve these apparent discrepancies.

The temporal sequence of changes in TH activity in LC and in NA metabolism in corresponding projection fields observed after combined lesions of RC and RD clearly differed. In fact, the increase in total DOPEG levels in NA-containing terminals was seen earlier than the augmentation of TH activity in the LC. The very fast increase in NA metabolite levels observed in hippocampus and cerebral cortex after raphe lesion may indicate that the inhibitory control of noradrenergic transmission exerted by 5-HT fibers is tonic in nature. As TH is rate controlling in the biosynthesis of NA, the raphe lesion-induced enzyme induction observed in LC may be viewed as a compensatory increase in the number of enzyme molecules in response to sustained activation of NA neuronal firing in order to cope with the high level of activity of NA neurons. It is of interest to note, in this respect, that a similar delayed induction of TH has been observed in the adrenal medulla after electrolytic lesion of the RC (Quick, Sourkes, Dubrovsky, & Gauthier, 1977).

Although combined lesions of RC and RD caused an increase in total DOPEG levels in noradrenergic projection fields, after individual lesions of these raphe nuclei a slight decrease in the concentrations of this NA metabolite was observed. A possible explanation for this apparent discrepancy may be that destruction of individual raphe nuclei is not sufficient to provoke the critical level of disinhibition necessary to trigger activation of the total NA system. The decrease in total DOPEG levels in NA terminals seen after individual raphe nuclei lesions may reflect an interruption of axonal flow between the LC and its terminal areas. Alternatively, since the hippocampus and cerebral cortex receive both NA and 5-HT terminals, these modifications may be indicative of an interaction at the presynaptic level between 5-HT and NA neurons.

Both investigations in which the 5-HT system was drastically deafferented showed that the increases in TH activity in LC and in total DOPEG levels in noradrenergic projection areas were transient. The transient nature of these increases despite continued depletion of 5-HT suggests the occurrence of compensatory mechanisms (e.g., development of 5-HT receptor supersensitivity or collateral sprouting) in LC that restore the normal function of NA neurons despite loss of the 5-HT afferents.

What ever the nature (facilitatory or inhibitory) of the serotonergic influence on LC noradrenergic cells, the present data suggest that the function of the ascending NA system originating from the LC might be largely dependent on its interaction with raphe 5-HT-containing neurons. This interaction is further supported by electrophysiological and biochemical investigations. In fact, it has been shown that the duration of depression of the firing rate of cortical neurons induced by NA is markedly diminished in rats with destroyed serotonergic systems or with depleted 5-HT content (Ferron, Descarries, &

Reader, 1982). Moreover, 5,7-DHT-induced destruction of 5-HT nerve terminals or depletion of 5-HT by p-chlorophenylalanine prevents the down-regulation of β-adrenergic receptors and the desensitization of NA-stimulated adenylate cyclase in the cerebral cortex induced by chronic antidepressant treatment (Racagni et al., 1983), thus indicating that 5-HT, per se, plays an important role in those homeostatic mechanisms that are operative in the adaptation of NA neurons to chronic antidepressants. Finally, acute administration of specific inhibitors of 5-HT uptake (e.g., indalpine, fluoxetine) has been shown to increase cerebral NA turnover (B. Scatton et al., unpublished observations, 1985).

The widespread projections of the LC throughout the neuraxis places this nucleus in a strategic position to influence numerous neurological and physiological functions. The present review has demonstrated that afferents to this nucleus, such as serotoninergic ones, play an important role in the transsynaptic regulation of NA neuronal activity in the LC. Thus, 5-HT-NA interaction appears to be pertinent to physiological and behavioral functions (e.g., learning and memory, regulation of affect, emotional behavior) in which NA has been suggested to play a key role.

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