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Activity of L-carnitine and L-acetylcarnitine on cholinoceptive neocortical neurons of the rat in vivo

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Summary. Carnitine and acetylcarnitine have been demonstrated to be present in the CNS and to be involved in cholinergic mechanisms, even if their exact role in neurotransmission is still unknown.

This microiontophoretic study was carried out on single cholinoceptive neurons of the somatosensory cortex in the rat in order to analyze the effects of L- and D-carnitine and L-acetylcarnitine on the spontaneous firing and the neuronal responses to some putative transmitters.

L-carnitine and L-acetylcarnitine increased the spontaneous discharge rate, while D-carnitine was found to be ineffective. L-acetylcarnitine clearly potentiated the cholinergic excitatory responses.

On the contrary, L-carnitine was found to reduce cholinergic responses in a great percentage of units and to inhibit L-acetylcarnitine-induced excitatory responses.

Atropine blocked the increase in firing rate induced by L-carnitine and L-acetylcarnitine, thus suggesting for both of them a muscarinic activity.

No interactions were observed between carnitines and GABA and glutamate.

These results show that carnitine and acetylcarnitine are stereospecific neuroactive compounds with a cholinomimetic activity. They may play a role in a modulatory system for the cholinoceptive cortical neuron.

Keywords: Carnitine, acetylcarnitine, microiontophoresis, acetylcholine, cerebral cortex.

Introduction

Carnitine and its derivatives have been known for their activity in the CNS intermediate metabolism for a long time (see data reviewed by Janiri and Tempesta, 1983).

The enzyme carnitine-acetyltransferase is evenly distributed throughout various cerebral regions (McCaman et al., 1966). However concentrations of the acetylated compound, acetylcarnitine, show regional differences, with the highest levels found in the hypothalamus (Bresolin et al., 1982).

Among the carnitine derivatives acetylcarnitine is thought to be involved in neural mechanisms, particularly of the cholinergic type. From its structural similarity to acetylcholine, acetylcarnitine has been postulated to have a role in cholinergic transmission (Sass and Werness, 1983). It has been hypothesized that acetylcarnitine may be synthesized by choline-acetyltransferase and catabolized by acetylcholinesterase (Hosein and Orzeck, 1964), while choline could be an alternative substrate for carnitine-acetyltransferase (White and Chen Wu, 1973). The recent finding that the drug is able to prevent the reduction of NGF binding sites on cholinergic neurons of senescent rats suggests a relationship between acetylcarnitine and the cholinergic system (Angelucci et al., 1989).

Microiontophoretic studies have demonstrated that carnitine and derivatives may influence the neuronal discharge rate. Falchetto et al. (1971) found that carnitine and acetylcarnitine are able to excite cat cortical units in an atropine-reversible manner and in doses much higher than those necessary for acetylcholine to induce excitations. They concluded that these compounds may act on synaptic membranes and stimulate muscarinic receptors, as well as putative neurotransmitters do, even though their release from presynaptic endings has yet to be proven (Falchetto et al., 1971).

The same cholinergic responses, blocked by atropine and enhanced by eserine were obtained in the cerebral cortex by Onofrj et al. (1983) with L-acetylcarnitine. Tempesta et al. (1982) observed increase in firing rate and potentiation of cholinergic and serotonergic transmission with application of D,L-acetylcarnitine to rat brainstem reticular neurons which are known to carry both muscarinic and nicotinic cholinergic receptors.

In a later study Tempesta et al. (1985) were able to confirm these effects in the brainstem for the L-form of acetylcarnitine, with additional evidence that the drug increased both excitatory and inhibitory responses to 5HT and its overall activity was blocked by dihydro-β-erythroidine rather than by atropine. They concluded that L-acetylcarnitine could act at a presynaptic level, at least with regard to serotonergic responses, and that nicotinic receptors may mediate the release of both acetylcholine and 5HT (Tempesta et al., 1985).

Forebrain projections to cerebral cortex represent a prominent cholinergic input (Mesulam et al., 1983) and are relevant to the understanding of neurological diseases with cognitive impairment, such as Alzheimer's dementia (Coyle et al., 1983). In the context of this postulated cholinergic pathophysiology, we have re-examined the electrophysiological actions of both carnitine and L-acetylcarnitine on rat neocortical neurons. The interactions between these compounds with one another and with acetylcholine and other neurotransmitters were studied in cholinoceptive units showing spontaneous or transmitter-evoked activity.

Table 1. Effects of microiontophoretic application of L-carnitine (L-Cn), D-carnitine (D-Cn) and L-acetylcarnitine (L-ACn) on the spontaneous firing rate of neurons in the somatosensory cortex of the rat

Drug	Total neurons	Excitation		No effect	
		n°	%	n°	%
L-Cn (+30 to +80 nA)	65	55	85	10	15
D-Cn (+30 to +80 nA)	29	4	14	25	86
L-ACn (+30 to +80 nA)	35	33	94	2	6

Material and methods

This microiontophoretic study was carried out on 32 Wistar rats of both sexes, weighing 200–250 gr.

The animals were anaesthetized with ethyl-urethane (1.8 gr/kg i.p.) and rectal temperature was maintained at 37 ± 0.5 °C by a thermostatically-controlled heating plate. The skull overlying the somatosensory cortex was removed and a small incision was made in the dura mater to allow insertion of the electrode assembly. The exposed neocortex was covered with paraffin oil.

Five-barreled glass micropipettes with 4–10 µm tips were used: the central recording barrel was filled with a 4M NaCl solution (D.C. resistance 4–10 Mohm); each of the other barrels contained the following aqueous solutions (D.C. resistance 20–50 Mohm): L-Carnitine chloride (0.5 M) pH 5, D-carnitine chloride (0.5 M) pH 5; L-Acetylcarnitine chloride (0.5 M) pH 5; acetylcholine chloride (0.5 M) pH 5; atropine sulphate (0.1 M) pH 5.5; monosodium-L-glutamate (0.5 M) pH 8; γ-aminobutyric acid (GABA) (0.5 M), pH 4.

One barrel of the micropipette was filled with 2 M NaCl solution in order to control for current effects.

The depth of penetration of the electrode was within 1.8 mm from the cortical surface (laminae 2–3, 4, and 5). The extracellular activity of single neurons was recorded and displayed using conventional techniques. Neurons showing both spontaneous and constant firing and steady responses to standard short pulses of acetylcholine (+30 to +60 nA) were selected and recorded. The microiontophoretic ejecting currents employed were +30 to +80 nA, while backing currents of − 15 nA were routinely used between applications. Glutamate was ejected by negative currents (− 5 to − 50 nA) and retained by positive currents (+10 − 15 nA). The intervals between drug application were kept constant as far as possible. Applications lasted 15–40 sec.

The neuronal firing rate was printed (Digital Printer-Pitman model) and plotted (Easterline Angus Speed Serve II).

Results

Effects of L-, D-carnitine and L-acetylcarnitine on spontaneous firing

The direct effects of L- and D-carnitine and L-acetylcarnitine on the spontaneous firing rate of single neurons were evaluated on 65, 29, and 35 neurons respectively (Table 1).

L-carnitine increased the mean frequency of discharge in about 85% of the

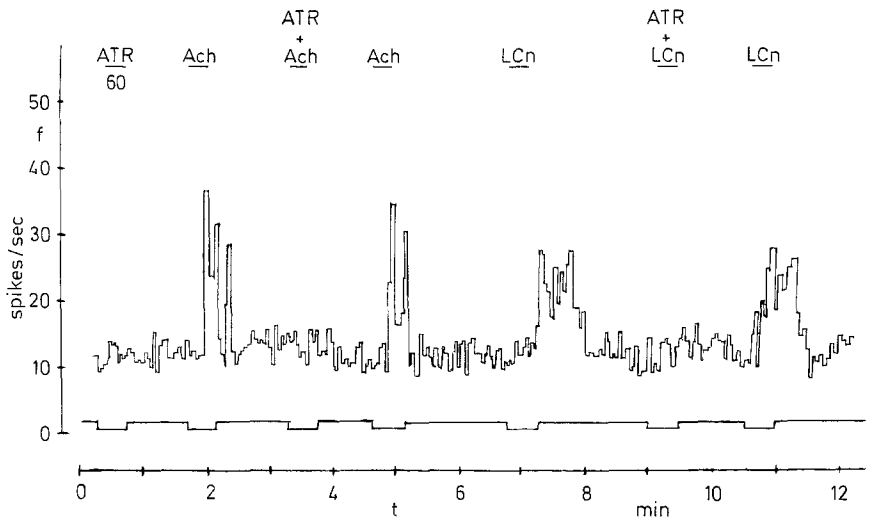

Fig. 1. Effects of iontophoretic applications of acetylcholine (Ach) and L-carnitine (LCn) on the firing rate of a single neuron of the rat somatosensory cortex. These excitatory responses were blocked by simultaneous administrations of atropine (ATR). In this and subsequent neuronal recordings phoretic currents are given in nano amperes (+60 nA); the mean firing rate in impulses/sec in successive 5 sec periods is plotted against time in minutes; iontophoretic applications of drugs are shown by horizontal bars

neurons while in the remaining 15% no modification in neuronal activity was recorded.

The responses were characterized by a fast rise in firing rate (50% increase within 5 sec.) and in about 65% they started at the end of the drug application outlasting the ejection period by 20–40 sec. In the remaining neurons (35%) we found earlier fast responses ending within the ejection period (15–40 sec.) (Fig. 1, 2).

The D-form was ineffective in about 86% of the units and in 14% it induced excitation (Fig. 2).

Almost all neurons (94%) were excited by L-acetylcarnitine. These responses were characterized by a very fast rise of the firing rate (100% increase within 5 sec.) with a latency of 5–10 sec. after the start of application. The effect lasted 20–60 sec.

The magnitude of the responses to L-carnitine and L-acetylcarnitine was directly related to the currents employed and the molarity of the solutions.

Effects of atropine on responses to acetylcholine, L-carnitine, L-acetylcarnitine

The effects of atropine on responses to acetylcholine, L-carnitine and L-acetylcarnitine are summarized in Table 2. Most responses to acetylcholine were fast excitations, with very short latency, generally lasting as long as the period of ejection or hardly outlasting it. The antagonistic action of atropine was tested

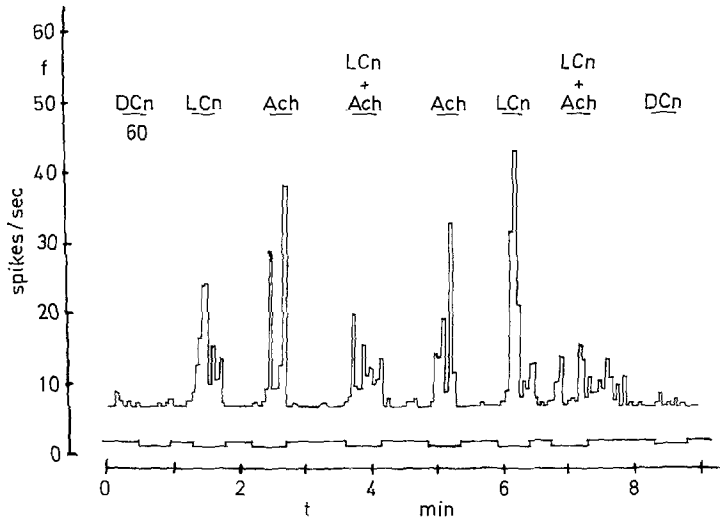

Fig. 2. Effects of iontophoretic applications of D- and L-carnitine (respectively DCn and LCn) and acetylcholine (Ach) on the firing rate of a single rat neocortical neuron. Simultaneous administrations of LCn and Ach reduced the excitatory responses to Ach

on 30 neurons. The drug did not display any effect on the firing rate. An inhibition of the cholinergic excitatory effects was observed in 28 out of 30 neurons (Fig. 1). Atropine could block these responses by approximately 67%.

As to the interaction between atropine and L-carnitine, the excitatory responses to the latter drug were blocked by the simultaneous application of the former by about 50% (Fig. 1) in 84% of the neurons tested.

Atropine could prevent the increase in firing rate induced by L-acetylcarnitine, reducing this effect by approximately 67% in 23 out of 25 neurons examined (Fig. 3). In all the trials performed with atropine all the neuronal responses to carnitines could be restored.

Effects of L-carnitine and L-acetylcarnitine on cholinergic responses

L-carnitine was found to reduce the cholinergic excitatory responses in 73% of the units tested (Table 3). This inhibition was dose-dependent and affected

Table 2. Effects of microiontophoretic application of atropine (ATR) on the neuronal responses to acetylcholine (ACh), L-carnitine (L-Cn) and L-acetylcarnitine (L-ACn) in the somatosensory cortex of the rat

Type of interaction	Total neurons	Potentiation		Inhibition		No effect	
		n°	%	n°	%	n°	%
ATR + ACh	30			28	93	2	7
ATR + L-Cn	25			21	84	4	16
ATR + L-ACn	25			23	92	2	8

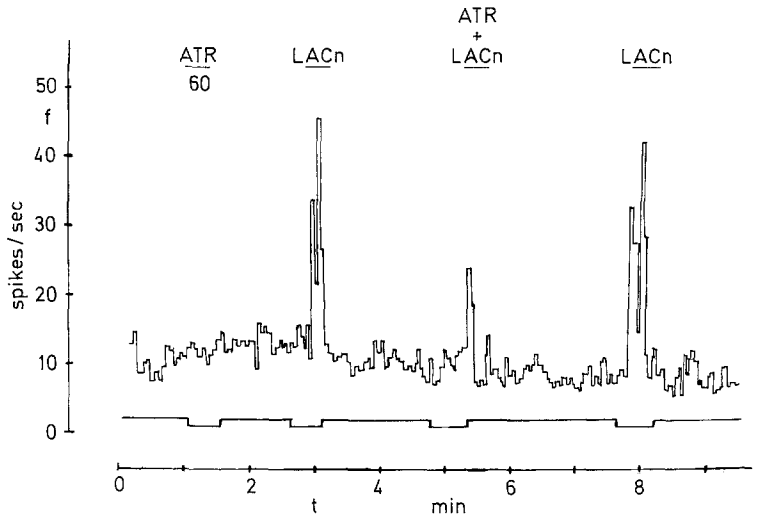

Fig. 3. Effect of iontophoretic applications of L-acetylcarnitine (LACn) on the firing rate of a single rat neocortical neuron. The response was inhibited by atropine (ATR)

more the intensity of the response rather than its duration which sometimes tended to be prolonged (Fig. 2). In certain cases L-carnitine produced a slight or no excitatory effect on the neuronal discharge rate; however, a blocking activity on cholinergic excitation was recorded. In the majority of neurons the early phase of the acetylcholine-induced excitation was particularly reduced by L-carnitine. On the contrary, a clear dose-dependent potentiation of the cholinergic responses was observed with L-acetylcarnitine in 85% of the units tested (Table 3). This effect involved both intensity and duration with no latency (Fig. 4). Full recovery of the control responses could be shown following all drug interaction tests.

Interactions between L-carnitine and L-acetylcarnitine

L-carnitine and L-acetylcarnitine were simultaneously applied to 20 neurons producing excitation (Table 3).

Table 3. Effects of microiontophoretic application of L-carnitine (L-Cn) and L-acetylcarnitine (L-ACn) on the neuronal responses to acetylcholine, and of L-Cn on L-ACn responses in the somatosensory cortex of the rat

Type of interaction	Total neurons	Potentiation		Inhibition		No effect	
		n°	%	n°	%	n°	%
ACh + L-Cn	37			27	73	10	27
ACh + L-ACn	26	22	85			4	15
L-ACn + L-Cn	20			18	90	2	10

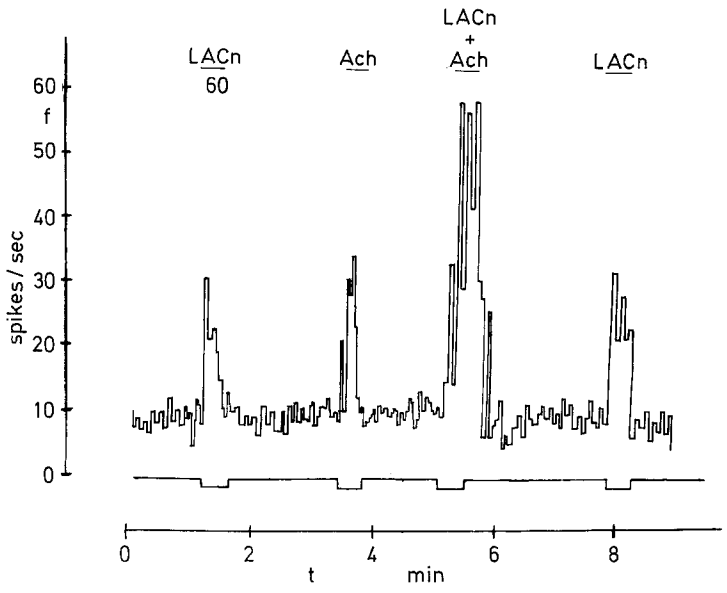

Fig. 4. Effect of iontophoretic applications of L-acetylcarnitine (LACn) and acetylcholine (Ach) on the firing rate of a single rat neocortical neuron. Simultaneous administrations of LACn and ACh potentiated the excitatory effect

In 18 units there was no excitatory effect; on the contrary there was a clear reduction or a reversible complete blockade of the expected increase in firing rate. This inhibition affected mostly the early phase of the response, which was reduced in both intensity and duration (Fig. 5).

Effects of L-carnitine and L-acetylcarnitine on responses to glutamate and GABA

The interactions between carnitines and amino-acid transmitters were merely additive.

In 16 units excited by L-glutamate and carnitines, the simultaneous administration of glutamate and either L-carnitine or L-acetylcarnitine evoked excitatory responses which were the simple addition of the neuronal responses to the single drugs separately applied (Fig. 6). The interaction between GABA and carnitines was additive as well. In fact 17 neurons inhibited by GABA showed reduced inhibitory effects to GABA when simultaneously tested with either L-carnitine or L-acetylcarnitine (Fig. 7).

Discussion

Previous data showed neural activity evoked mainly by the L-form of acetylcarnitine in comparison with the D-enantiomer (Tempesta et al., 1985). Also in another microiontophoretic study performed in the cerebral cortex, the L-form was employed with evidence of pharmacologically specific effects (Onofrj

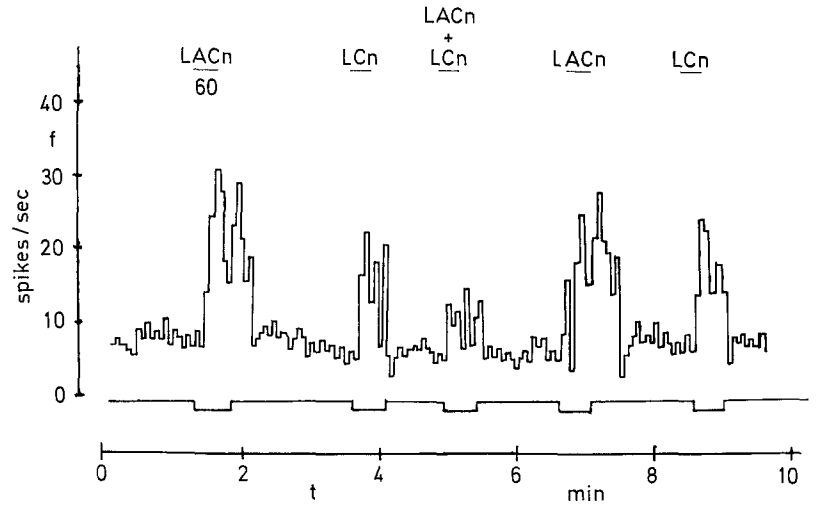

Fig. 5. Effect of iontophoretic applications of L-carnitine (LCn) and L-acetylcarnitine (LACn) on the firing rate of a single rat neocortical neurone. Simultaneous administrations of LCn and LACn reduced the excitatory effect of the latter drug

et al., 1983). Thus in our experiments we have used L-acetylcarnitine which was shown to possess definite excitatory properties on neuronal cells. Besides, we have tested carnitine for stereospecificity; the L-form was found to increase spontaneous firing, while the D-compound was almost inactive. This is in agreement with the results of other authors, who employed the microiontophoretic technique (Falchetto et al., 1971), or biochemical methods (Strack and

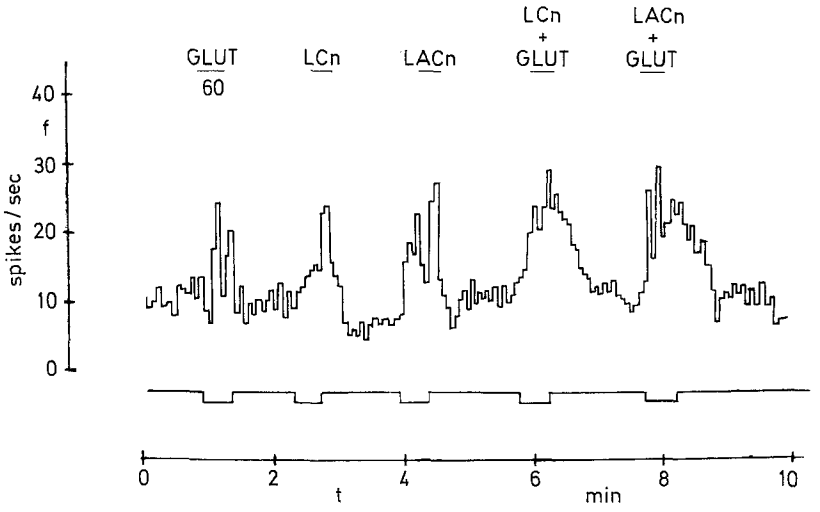

Fig. 6. Effect of iontophoretic applications of glutamate (GLUT), L-carnitine (LCn) and L-acetylcarnitine (LACn) on the firing rate of a single rat neocortical neuron. Simultaneous administrations of either LCn or LACn and GLUT evoked merely additive effects

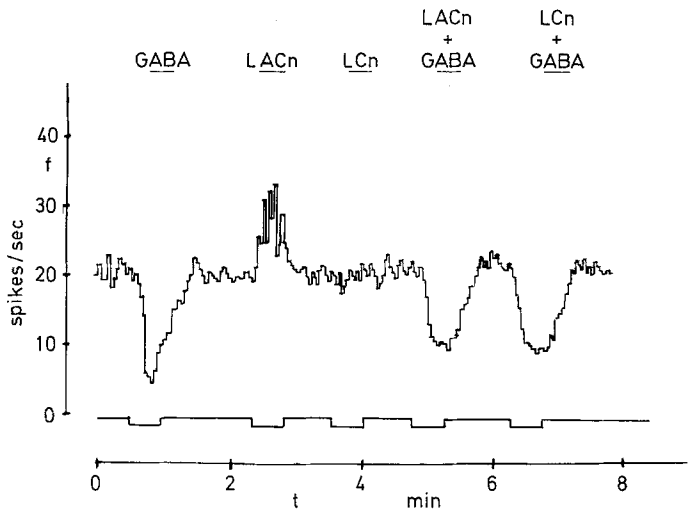

Fig. 7. Effect of iontophoretic applications of GABA, L-acetylcarnitine (LACn), and L-carnitine (LCn) on the firing rate of a single rat neocortical neuron. Simultaneous administrations of either LCn or LACn and GABA evoked additive effects

Lorenz, 1966; Thomitzek et al., 1966). All of them concluded that the effects displayed by carnitine and derivatives could be selectively attributed to the L-forms.

In the present study excitations induced by L-carnitine and L-acetylcarnitine were fast in rise and without significant latency. Falchetto et al. (1971) found a slow rate of onset of excitation in cortical neurons. Tempesta et al. (1982, 1985) observed long latency of most neuronal responses to D, L- and L-acetylcarnitine in the rat brainstem. This discrepancy between the latencies of the observed effects can depend upon experimental conditions, such as drug diffusion and pipette characteristics. Nevertheless, the heterogeneity of the neuronal populations examined may partly underlie the difference between the results obtained in the brainstem and those in the cerebral cortex: brainstem cholinoceptive neurons seem to be excited by acetylcholine mainly by nicotinic receptors (Bradley and Dray, 1972), while cholinergic responses of most cortical units have been demonstrated to be of the muscarinic type (Curtis, 1976). Besides the complexity of the neuropil at the medullary-pontine reticular formation, where cholinergic neurons appear to exert a presynaptic control on monoaminergic terminals, makes brainstem a peculiar recording area for assessing the effects of acetylcholine (Butcher and Woolf, 1982).

Cholinergic excitations were almost fully blocked by atropine and this fact is in agreement with the existence of a predominant population of muscarinic receptors throughout all cortical areas (Curtis, 1976). Moreover, brain lesions of Alzheimer's dementia include subcortical regions rich in cholinergic neurons projecting to widespread muscarinic cholinoceptive field in the cerebral cortex (Coyle et al., 1983).

Both effects of carnitines on the spontaneous firing of cortical neurons appeared to be cholinergic. However, the demonstration of opposite-sign effects displayed by L-carnitine and L-acetylcarnitine on cholinergic responses is particularly interesting. L-acetylcarnitine evoked excitatory responses and potentiated cortical responses to acetylcholine in an atropine-reversible manner. L-acetylcarnitine potentiated responses to acetylcholine at the brainstem reticular formation as well, but this effect was not atropine-reversible (Tempesta et al., 1985). Also the effects of L-carnitine could be reversed by atropine, though to a lesser extent than those of acetylcholine and L-acetylcarnitine. However, L-carnitine affected the neuronal responses to these compounds in a direction opposite to that of its own responses.

This fact leads us to postulate a modulatory function for L-carnitine, an endogenous compound, in the cortical cholinergic transmission, according to the concept of modulation as a form of neuroregulation (Dismukes, 1979; Elliott and Barchas, 1979). Endogenous modulatory mechanisms have been proven to exist in the cerebral cortex, and to play a relevant role in the neuronal circuitry. Local modulation of neurotransmitter release involving either autoreceptors or presynaptic control by other transmitters has been described by many authors (see data reviewed by Chesselet, 1984). Opioid peptides inhibiting the discharge rate of cortical units may reduce the effect of other inhibitory opioids (Janiri et al., 1988). This phenomenon has been interpreted as an endogenous activity of the agonistic-antagonistic type, namely a modulatory mechanism, at the postsynaptic level (Janiri et al., 1988). In a similar manner, it seems paradoxical that L-carnitine and acetylcholine both excite cortical cells, but their simultaneous application leads to an inhibition of both excitatory responses. As well as in the case of the cortical opioid peptide system, L-carnitine could reduce responses to acetylcholine or to L-acetylcarnitine even when there was no effect or a slight effect on the spontaneous discharge rate.

It is difficult to conclude by the paradigm used whether the mode of action of L-carnitine is at a pre- or postsynaptic site. Falchetto et al. (1971) suggested that carnitines in the cerebral cortex do not affect the release of excitatory transmitters. Tempesta et al. (1985) hypothesized that the mechanism of action of L-acetylcarnitine in the brainstem could be mediated by presynaptically-located receptors. However, the present results on both carnitines cannot account for either mechanisms.

It is conceivable that L-carnitine could change resting membrane potential and increase spontaneous discharge. Thus the driving force for acetylcholine effects would be decreased and the cholinergic excitation would be lost. However, such hypothesis cannot explain the fact that L-acetylcarnitine potentiated cholinergic effects and that both L-carnitine and L-acetylcarnitine, in doses not liable to induce a depolarization block, added their activity to that of L-glutamate without any reduction of the effect's magnitude.

This additive interaction between carnitines and amino-acid transmitters, GABA included, was also previously observed at the brainstem level (Tempesta et al., 1985).

In order to ascertain the physiological significance of the hypothesis that endogenous compounds, such as carnitines, could regulate the firing of cholinoceptive cortical neurons with different rank and potency of receptor interaction, further studies are required.

As L-acetylcarnitine has been found to improve cognitive deficits in aged rats (Angelucci et al., 1988), the demonstration that these substances might be released as endogenous neuroregulators would be of relevance. In fact the nerve cell loss associated with aging or degenerative diseases in animals as well as in humans could coexist with an impaired balance of synaptic regulatory systems (Bowen et al., 1976; Rossor, 1982).

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