Central Cholinergic Modulation of Carrageenin-Induced Pedal Inflammation in Rats

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Abstract: Possible central cholinergic modulation of acute peripheral inflammation was investigated in rats, adopting the carrageenin-induced acute pedal edema as the experimental model. Intracerebroventricularly (icv) administered acetylcholine (Ach) and tremorine, a central cholinomimetic agent, significantly augmented the inflammation, whereas hemicholinium, which inhibits Ach synthesis, attenuated the edema. Paradoxically, icv administered atropine sulphate induced an Ach like pro-inflammatory effect and also failed to affect the Ach action. The atropine-induced inflammation promoting effect was annulled after hemicholinium pretreatment, suggesting that it was dependent on the integrity of central cholinergic function. The pro-inflammatory effect of icv Ach was negated after peripheral administration of atropine ethoiodide. It is suggested that the carrageenin inflammation promoting effect of centrally administered Ach is due to enhanced peripheral cholinergic activity.

The cascade of events, mediated by various autacoids, culminating in inflammation, and the different factors which serve to sustain or limit the inflammatory response, are fairly well defined (1). Unlike the peripheral influences modulating inflammation, little is known about the role of the central nervous system (CNS), though there are indications that a central modulatory influence might exist. It has been very aptly remarked that paucity of relevant data characterizes the question of putative involvement of the CNS in peripheral inflammation (2). With a view to bridge this lacunae, a critical investigation of the role of the CNS in peripheral inflammation has been undertaken. In this communication we report the role of the central cholinergic system on carrageenin-induced pedal edema in rats.

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Materials and Methods

The studies were conducted on inbred Wistar strain rats (120–180 g) of either sex obtained from the Institute animal house. The rats were housed in colony cages at an ambient temperature of 25±2°C and fed on standard Hind Lever chow. Experiments were conducted at this ambient temperature, between 9 a. m. and 2 p. m. Pedal inflammation was induced by injecting 0.1 ml of 1% carrageenin suspension in 0.9% saline, below the plantar aponeurosis. The increase in paw volume, after injection of the phlogistic agent, was taken as the index of inflammation and was recorded by means of a mercury plethysmograph, before and at hourly intervals for 4 h after carrageenin administration (3). The increase in paw volume was expressed in units, each unit representing 1 cm (volume = 0.075 ml) length of the displaced mercury.

Intracerebroventricular (icv) cannulation was performed in pentobarbitone sodium (40 mg/kg, ip) anesthetized rats. Indwelling cannulae were inserted into the right lateral ventricle stereotaxically (4). Drugs for icv administration were dissolved in 10 µl of artificial cerebrospinal fluid (CSF). Control animals received an equivalent volume of artificial CSF through the same route.

The following drugs were used in the study: acetylcholine chloride (Sigma), tremorine hydrochloride (Sigma), hemicholinium-3 (Sigma), atropine sulphate (Boehringer) and atropine ethoiodide (prepared by the Department of Medicinal Chemistry of this Institute). The doses, pretreatment times and the routes of administration used, are indicated in the appropriate tables.

Statistical analysis was done by the Student's t test.

Results and Discussion

The results are summarized in Fig. 1 and Tables I and II. Centrally (icv) administered acetylcholine (Ach) and tremorine augmented carrageenin-induced pedal edema. On the contrary, hemicholinium

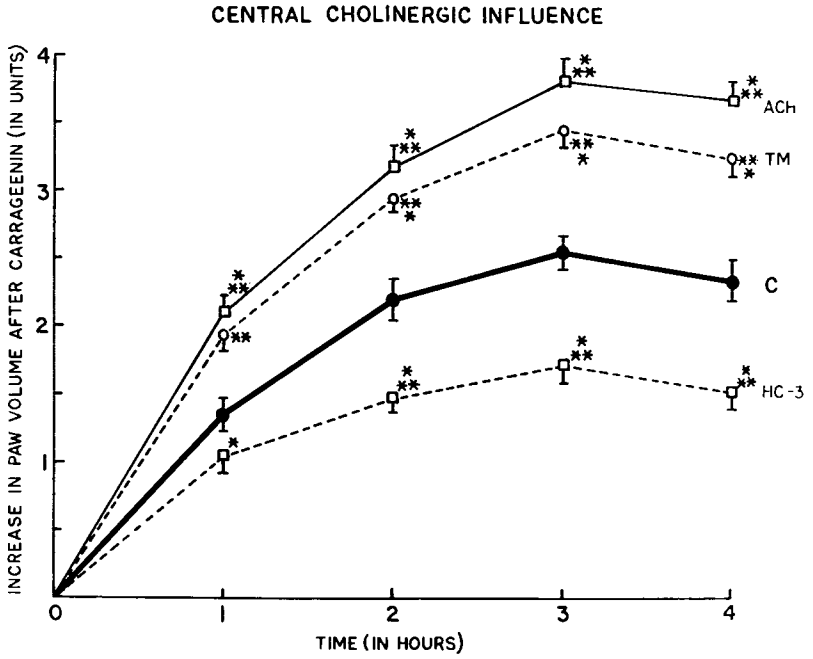

Fig. 1 Effect of icv administered acetylcholine (Ach), tremorine (TM) and hemicholinium (HC-3) on carrageenin-induced pedal edema in rats. C: Control (artificial CSF). Vertical lines indicate S.E.M. P values: *<0.05, **<0.01, ***<0.001.

Table I. Effects of icv Administered Cholinergic Agonists and Antagonists on Carrageenin-Induced Pedal Edema in Rats.

Groups	n	Dose (μg)	Pretreatment time (min)	Increase in paw volume in units (Mean ± S.E.M.) 1 h	2 h	3 h	4 h
1. Control (artificial CSF)	7	–	–	1.41 ± 0.11	2.31 ± 0.19	2.72 ± 0.18	2.65 ± 0.17
2. Ach	10	50	15	2.09 ± 0.11[c]	3.17 ± 0.14[b]	3.81 ± 0.18[c]	3.67 ± 0.15[c]
3. Atropine sulphate	8	10	15	2.17 ± 0.29[a]	3.66 ± 0.32[b]	4.02 ± 0.32[b]	3.82 ± 0.33[b]
4. Atropine sulphate + Ach	5	10 + 50	30, 15	2.16 ± 0.13[ns]	2.90 ± 0.19[ns]	3.44 ± 0.24[ns]	3.22 ± 0.17[ns]
5. Hemicholinium (HC)	13	20	45	1.03 ± 0.08[b]	1.47 ± 0.08[c]	1.71 ± 0.08[c]	1.52 ± 0.1[c]
6. HC + Atropine sulphate	4	20 + 10	60, 15	0.97 ± 0.04[d]	1.50 ± 0.16[c]	1.77 ± 0.16[e]	1.70 ± 0.14[e]

Statistical significance in relation to:
Control group (1): a <0.05; b <0.01; c <0.001
Ach group (2): [ns] >0.05
Atropine group (3): [d] <0.01; [e] <0.001

Table II. Effect of Atropine Ethoiodide (ip) on icv Ach-Induced Increase in Carrageenin Pedal Edema in Rats.

Groups	n	Dose (kg^{-1})	Pretreatment time (min)	Route	Increase in paw volume in units (Mean ± S.E.M.) 1 h	2 h	3 h	4 h
1. Control (artificial CSF)	14	–	–	icv	1.36 ± 0.12	2.20 ± 0.15	2.5 ± 0.12	2.36 ± 0.15
2. Ach	10	50 μg*	15	icv	2.09 ± 0.11[c]	3.17 ± 0.14[c]	3.81 ± 0.18[c]	3.67 ± 0.15[c]
3. Atropine ethoiodide	5	5 mg	60	ip	1.78 ± 0.15	2.30 ± 0.31	2.42 ± 0.27	2.2 ± 0.26
4. Atropine ethoiodide + Ach	6	5 mg 50 μg*	75 15	ip icv	1.43 ± 0.13[d]	1.91 ± 0.15[d]	2.23 ± 0.15[d]	2.15 ± 0.09[d]

* = Total dose given per rat icv
Statistical significance in relation to:
Control group (1): [c] <0.001
Ach group (2): [d] <0.001

(HC), which inhibits Ach synthesis by inhibiting uptake of choline into cholinergic neurons, caused a significant decrease in the degree of pedal inflammation as compared to artificial CSF administered controls (Fig. 1). Paradoxically, icv administered atropine sulphate augmented the inflammatory response (Table I). Since Ach and atropine produced a similar pro-inflammatory effect throughout the period of observation, some drug interaction studies were conducted. Atropine pretreatment did not affect Ach response. The pro-inflammatory effects of Ach or atropine, administered icv, given individually or in combination, were not significantly different from each other. The inflammation enhancing effect of atropine was, however, completely antagonized in hemicholinium treated rats (Table I). Peripheral (ip) administration of atropine ethoiodide, a quaternary ammonium *m*-cholinolytic agent incapable of crossing the blood-brain barrier, markedly antagonized the pro-inflammatory effect of icv administerd Ach (Table II). Centrally administered Ach, tremorine, hemicholinium and atropine sulphate, and ip administered atropine ethoiodide, had no effect on paw volume in non-carrageenin rats in the doses used.

The carrageenin model of acute pedal inflammation in rats was selected because of its reproducibility and the fact that the edema depends entirely on local inflammatory reaction devoid of antigenic properties (1). Furthermore, an excellent correlation exists between the anti-phlogistic and clinical anti-arthritic effects of a host of drugs (5).

Ach and tremorine, a central cholinomimetic agent, significantly enhanced carrageenin-induced pedal edema, on central administration, throughout the 4 h observation period. On the contrary, hemicholinium which inhibits Ach synthesis significantly reduced the edema on icv administration. These results indicate that the central cholinergic system, in rats, exerts a pro-inflammatory modulatory effect on acute peripheral inflammation, in contrast to the modulatory inhibitory effect of central norepinephrine (6).

It is well accepted that central neurotransmitter systems do not function in isolation but interact to modulate homeostasis in a balanced manner. Intensive inter-connections are known to exist between noradrenergic and cholinergic neurons in several rat brain areas (7). Cholinergically activated noradrenergic inhibitory systems are present in the cerebral cortex of rats (7). Central cholinergic hyperactivity, following reserpine-induced depletion of catecholamines, is consonant with the postulated reciprocal inhibitory interaction between these two neurotransmitter systems. It is of interest to note that reserpine has been shown to induce a pro-inflammatory effect on carrageenin edema, after icv administration (6). Hemicholinium has been reported to enhance central noradrenergic activity (8) and the observed anti-inflammatory effect of the drug might also be attributable to this activity.

Centrally administered atropine not only did not affect Ach action, it produced an Ach-like inflammation augmenting effect. This apparently para-

doxical action requires elucidation. Convincing experimental evidence suggests that presynaptic muscarinic receptors autoregulate Ach release in the CNS (9). Atropine has been shown to induce a marked and long lasting increase in Ach output, both *in vitro* and *in vivo* (9). Since the pro-inflammatory effect of centrally administered atropine was negated following treatment with hemicholinium, it is reasonable to assume that the atropine effect was a consequence of presynaptic rather than postsynaptic blockade manifested as cholinomimetic instead of cholinolytic action of the drug.

The pro-inflammatory effect of Ach was markedly inhibited after peripheral administration of atropine ethoiodide, a quaternary ammonium salt with poor access into the brain. This indicates that the observed effect of centrally administered Ach involves peripheral muscarinic receptors. Increased central cholinergic activity is known to be reflected by enhanced peripheral cholinergic activity. Central cholinergic activation induces peripheral vasodilatation and hypotension, through peripheral cholinergic activation (10). Cholinergic vasodilatation has been associated with the early phase of the inflammatory response (2). Cholinoceptors, when activated, enhance the release of inflammation-inducing mediators, including histamine and SRS-A, an effect blocked by atropine (11). Furthermore, cholinomimetics activate guanylate cyclase to increase cGMP levels, which then enhances the release of inflammatory autacoids (12). Atropine, and not ganglion blocking agents, inhibits cGMP mediated release of inflammatory mediators (12). It is likely that central cholinergic activation, following icv administration of Ach, tremorine and atropine, results in peripheral cholinergic stimulation leading to vasodilatation and the release of inflammatory mediators, an effect blocked by atropine ethoiodide. The observed effect of centrally administered Ach, tremorine, hemicholinium and atropine, on peripheral inflammation, does not appear to be due to any peripheral leakage effect because none of these agents produced any *per se* effect when administered ip in doses used for icv administration (13). Peripherally administered atropine ethoiodide, likewise, failed to affect the carrageenin edema to any appreciable extent, lending credence to the postulate that presynaptic muscarinic receptor modulation of Ach release is mainly a CNS phenomenon (9).

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