Induction of Hepatic Tyrosine Aminotransferase mediated by a Cholinergic Agent

TYROSINE aminotransferase in the rat liver is subject to regulation by a number of hormones and amino-acids[1-3]. Recent work has indicated that the sympathetic neurotransmitter norepinephrine suppresses the synthesis of tyrosine aminotransferase by forming a complex with the pyridoxal 5′-phosphate cofactor (refs. 4–6 and I. B. B., manuscript submitted for publication). Evidence suggesting mediation of this effect by peripheral autonomic nerves has been presented[7]. Because many of the physiological consequences of sympathetic stimulation are antagonized by parasympathetic discharge with acetylcholine release, the effect of cholinergic agents on enzyme regulation was examined. I report here that the cholinergic agent carbachol (carbamylcholine) induces hepatic tyrosine aminotransferase and that this induction is antagonized by the cholinergic blocking agent atropine.

Adrenalectomized, female, Sprague–Dawley rats (160–180 g) were subjected to a diurnal lighting schedule previously described[4] and offered one per cent NaCl solution *ad libitum*. Animals were used 4 to 6 days after adrenalectomy and were fasted from the onset to the termination of each experiment. All studies were performed between 0800 h and 1200 h.

The effects of administered acetylcholine are evanescent, presumably because of the rapid hydrolysis by acetylcholinesterase and non-specific cholinesterases. The choline ester carbachol, which is resistant to these enzymes, was therefore used to mimic acetylcholine. After treatment, rats were killed by a blow to the head and livers were rapidly removed and frozen. Livers were homogenized in isotonic KCl solution and tyrosine aminotransferase activity was assayed by a modification of the method of Diamondstone[8].

Carbachol administration resulted in a two-fold rise in tyrosine aminotransferase activity within 2 h (Fig. 1). Bethanecol, another cholinergic agent, caused a similar

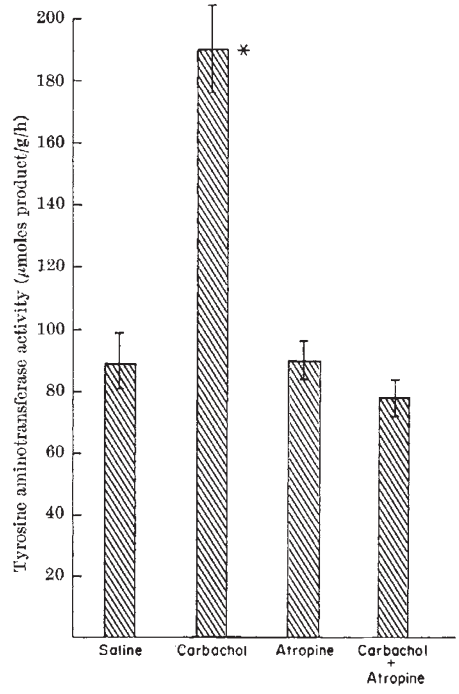

Fig. 1. Specificity of tyrosine aminotransferase activity elevation after carbachol. Rats were injected with carbachol 0·75 mg/kg at 0900 h and/or with atropine 10·0 mg/kg at 0845 h. Controls were treated with saline at appropriate times. All injections were intraperitoneal in a volume of 1 ml. All rats were killed at 1100 h. Results are expressed as mean ± *S.E.* (brackets). * Differs from all other groups at $P < 0.001$.

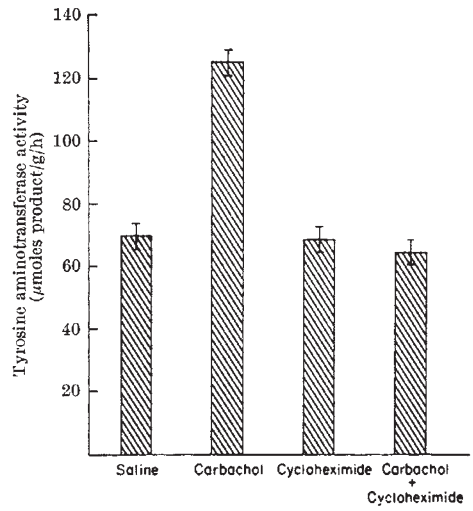

Fig. 2. Induction of tyrosine aminotransferase by carbachol. Rats were treated with carbachol as in Fig. 1, and/or with cycloheximide 20 mg/kg intraperitoneally 15 min earlier. Controls were treated with saline. Animals were killed 2 h after carbachol administration. Results are expressed as mean ± *S.E.* (brackets). The group treated with carbachol above differs from all other groups at $P < 0.001$.

increase in enzyme activity. To determine whether the increased enzyme activity was the result of cholinergic receptor stimulation, additional groups of rats were also treated with atropine. This compound, which competitively antagonizes the effects of cholinergic agents, blocked the rise in tyrosine aminotransferase activity after carbachol. Atropine alone, in the doses used, did not alter enzyme activity (Fig. 1).

Enzyme activity may increase either through activation of existing molecules or as a result of an increase in the amount of enzyme protein. To distinguish between these alternatives, rats were treated with the inhibitor of protein synthesis, cycloheximide, before receiving carbachol. Cycloheximide prevented elevation of tyrosine aminotransferase by carbachol (Fig. 2), indicating that on-going protein synthesis is necessary for this rise in enzyme activity.

Induction of tyrosine aminotransferase by carbachol and blockage of this effect by atropine suggests that this induction is mediated by a cholinergic receptor. The effect of this cholinergic agent is opposite to that previously described for the sympathetic neuromediator, norepinephrine. Norepinephrine suppresses tyrosine aminotransferase synthesis by binding pyridoxal 5′-phosphate, whereas carbachol induces the enzyme. Thus, although these two neuromediators may be operating at different sites, this enzyme system provides a molecular model for the recognized antagonism of the sympathetic and parasympathetic nervous systems.

The locus of action of carbachol has not been determined. Because acetylcholine has been associated with the release of pancreatic insulin[9] (a known inducer of tyrosine aminotransferase[10]), the drug may be acting through hormonal mediation.

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