Short communications

The role of brain acetylcholine in the pressor response to centrally injected neostigmine

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In unanaesthetized rats, neostigmine (2·5 μg) injected into the cerebral ventricles produces a rise in arterial blood pressure. The effect on the rise was examined after lowering the acetylcholine (ACh) level of the brain by hemicholinium (HC-3) similarly injected.

The pressor response was not significantly altered 2 h after injection of 50 μg HC-3 when the ACh level was reduced by 54%. It was greatly attenuated 2 h after injection of 75 μg when the ACh level was reduced by 76%. However, a similar attenuation was observed as early as 30 min after this injection when the ACh level was reduced by 18% only.

It is concluded that the pressor response is not mediated by undestroyed ACh but is a direct central effect of neostigmine.

Central administration of various cholinomimetic drugs evoke changes in arterial blood pressure and heart rate (Armitage & Hall, 1967; Brezenoff & Jenden, 1969; Brezenoff & Wirecki, 1970). It was recently reported that microinjections of the cholinesterase inhibitor, neostigmine, into the caudal hypothalamus produced a delayed pressor response (Brezenoff, 1972). In view of the presence of acetylcholine (ACh) in the hypothalamus (MacIntosh, 1941; Lederis & Livingston, 1969), these findings suggest the possibility of a role for brain ACh in cardiovascular regulation. The present study was designed to test this possibility by examining the centrally-induced cardiovascular effects of neostigmine following reduction of brain ACh levels with hemicholinium-3 (HC-3) (Slater, 1968).

Method.—Adult, male Sprague-Dawley rats weighing 250–275 g were used in this study. Under ketamine anaesthesia, guides for injections into the cerebral ventricles (23 gauge stainless steel tubing) were directed toward the lateral ventricle and permanently fixed to the skull with dental cement. The guide was then plugged with a 30 gauge stilette and the rat was allowed at least one week to recover from surgery. Solutions were injected into the ventricle via a 30 gauge injection cannula inserted through the guide. The injection cannula was connected via polyethylene tubing to a microlitre syringe. Drugs were dissolved in 0·9% w/v NaCl solution (saline) and injected in a volume of 10 μl.

Two days prior to the day of the experiment the animals were prepared for chronic direct recording of arterial blood pressure. Under ether anaesthesia a section of polyethylene tubing (P.E. 50), prefilled with saline/heparin solution, was inserted into the common carotid artery. The catheter was brought out at the back of the neck and sealed with a 23 gauge stilette.

On the day of the experiment the rats were pretreated with injections of either saline or HC-3 solutions into the lateral ventricle. Neostigmine was injected by the same route 2 h following pretreatment, unless otherwise specified.

Recording of arterial blood pressure was begun approximately 5 min before neostigmine or saline was administered. For this purpose the rat was restrained in a cloth wrapping and the indwelling arterial catheter was connected to a Statham pressure transducer PAC-23) coupled to a Gilson polygraph.

Arterial blood pressure was monitored for 10 min following administration of neostigmine. At the end of this period the rat was killed by decapitation and the brain was removed, weighed and homogenized in 5 ml of perchloric acid (7·5 N). The homogenate was assayed for ACh by the gas-chromatographic technique described by Hanin, Massarelli & Costa (1972).

Results.—Basal systolic and diastolic blood pressure in 4 control rats averaged $156 \pm 3/123 \pm 6$ mmHg (mean ± S.E.M.). Similar values were obtained in 8 other animals when recorded 2 h after injection into the lateral ventricle of 50 or 75 μg of HC-3.

Injections of neostigmine, 2·5 μg into the lateral ventricles of the control rats evoked a rise in arterial blood pressure of $61 \pm 7/30 \pm 8$ mmHg. This response began approximately 1 min after drug administration and became maximal during the

TABLE 1. *Effect on arterial blood pressure (BP) on injections of neostigmine (2·5 μg) into the cerebral ventricle*

	Pre-treatment with HC-3 (μg)			
		2 hours		30 min
	Saline	50 μg	75 μg	75 μg
Control BP (mmHg)				
(systolic)	156±3	159±2	153±3	145±3*
(diastolic)	123±6	126±2	121±2	108±2
Rise in BP after neostigmine				
(systolic)	61±7	55±2	34±2†	29±7†
(diastolic)	28±8	34±2	34±6	24±5
ACh (nM/g)	10·0±7	4·6±1·1†	2·4±4†	8·2±2·2

* $P<0.05$ (compared to saline controls); † $P<0.01$.

next 3–4 minutes. The increased pressure was maintained for the duration of the experiment (10 minutes). Similar injections of saline did not affect arterial blood pressure.

As indicated in Table 1, pretreatment for 2 h with 50 μg of HC-3, injected intraventricularly, did not significantly alter the pressor response to neostigmine in 4 experiments. Brain ACh levels in these animals, however, averaged 54% less than the 10·0±0·7 nM/g found in the control rats ($P<0.01$). Two hours pretreatment with 75 μg of HC-3 caused a 50% reduction in the pressor response to neostigmine ($P<0.05$). This was accompanied by a 76% reduction in brain ACh concentration. In four other animals, however, a similar inhibition of the pressor response was observed when neostigmine was administered 30 min after 75 μg of HC-3. Brain ACh levels at this time were reduced only 18% compared to control.

Discussion.—Systemic administration of various cholinesterase inhibitors causes a pressor response in the rat (Dirnhuber & Cullumbine, 1955; Varagić, 1955). It has been suggested that this effect is the result of a centrally mediated increase in sympathetic activity (Varagić, 1955; Varagić & Vojvadić, 1962). This suggestion is supported by the observation that intrahypothalamic injections of physostigmine (Brezenoff & Jenden, 1969) and neostigmine (Brezenoff, 1972) produced a rise in arterial blood pressure. The present study has demonstrated that injection of neostigmine into the cerebral ventricles evokes a pressor response in the unanaesthetized rat.

The pressor response to neostigmine was not impaired by doses of HC-3 which caused a 54% reduction in brain ACh. Although the response was reduced following the higher dose of HC-3, the inhibition does not appear to be the result of diminished brain levels of ACh. Thus, a similar inhibition of the hypertensive response to neostigmine was seen 30 min after intraventricular injection of 75 μg HC-3, although brain ACh levels at this time were still 82% of control. High doses of HC-3 are known to block postsynaptic cholinergic receptors (Schueler, 1960), and it is likely that this action is responsible for the reduced pressor response to neostigmine following pretreatment with 75 μg of HC-3.

Inhibition of cholinesterase does not appear to be responsible for the pressor response to neostigmine. The onset of the pressor effect occurred approximately 1 min after neostigmine administration and it is unlikely that significant cholinesterase inhibition could occur in this time. In addition, reduction of brain ACh levels to 50% of control failed to modify the cardiovascular effect of centrally administered neostigmine. This latter argument also makes it unlikely that the pressor response results from an action of neostigmine on cholinergic nerve terminals. Thus, the most likely explanation for the centrally mediated pressor response is a direct postsynaptic action of neostigmine.

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