STRAIN DIFFERENCES IN THE INHIBITION BY HEMICHOLINIUM-3 OF THE PRESSOR RESPONSE TO INTRAVENTRICULAR INJECTION OF NEOSTIGMINE IN RATS

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The pressor response to intracerebroventricular (i.c.v.) injections of neostigmine was examined in three groups of Sprague-Dawley rats (MFS, CS and CFE) obtained from different breeders and in Long-Evans rats (MLE) obtained from one of the same breeders. All rats responded with a rise in blood pressure to i.c.v. injection of neostigmine, although the CFE strain was 6-fold less sensitive to the drug than were the other strains.

Prior i.c.v. injection of hemicholinium-3 (HC-3; 0.4 μg) reduced by more than 50% the pressor response in the two strains obtained from the same breeder (MFS and MLE) and caused an 11% reduction in the CS strain. In contrast, the pressor response in CFE rats was not affected by as much as 40 μg of HC-3, although this dose decreased the response to neostigmine by 47-66% in the other three strains.

Certain anticholinesterase agents cause a centrally mediated rise in arterial blood pressure following their systemic administration in the rat (Dirnhuber & Cullumbine, 1955; Varagić, 1955; Varagić & Vojvadić, 1962). More recently, it has been demonstrated that both physostigmine (Brezenoff, 1973a) and neostigmine (Brezenoff, 1973b) evoke a pressor response following injection into rat cerebral ventricles.

It was originally reported (Brezenoff, 1973b) that the pressor response to neostigmine was not abolished by prior reduction of brain acetylcholine with hemicholinium-3 (HC-3), suggesting a direct central action of neostigmine. The results of subsequent experiments in this laboratory, however, indicated that as much as 60% of the response could be abolished by HC-3 administration. In the interim between the two studies, the animal supplier had moved to new facilities and had begun a new colony of Sprague-Dawley derived rats obtained from a different source from the old one. The new colony was started because of a respiratory ailment which developed in the original colony at about the time of the initial study.

In view of the diverse results obtained in the two groups of animals, the experiments were repeated in rats obtained from several different sources to establish whether or not strain or supply differences were a contributing factor. The results of these experiments are described in this communication and indicate marked differences between certain strains in their central responses to neostigmine and HC-3.

Methods Four groups of rats were employed in these studies. Three groups were Sprague-Dawley derived: MFS, Marland Farms (Hewlett, N.J.); CS, Camm Research Institute (Wayne, N.J.); CFE, Carworth Farms (New City, N.Y.). Additionally, a group of Long-Evans derived rats was studied (MLE, Marland Farms, Hewlett, N.J.). All animals were adult males weighing 260-280 g.

Under methohexitone anaesthesia, a 23 gauge stainless steel injection guide was directed toward a lateral cerebral 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 by 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.

One day prior to the day of the experiment the animals were prepared for chronic 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.

To record arterial blood pressure, 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. All experiments were performed in the

unanaesthetized animal. Student's t test was used to determine significance.

Results The predrug levels of systolic blood pressure in the CFE strain (176 ± 4 mmHg; mean ± s.e. mean) was higher than either the MLE (158 ± 4 mmHg; $P < 0.02$) or CS (164 ± 3 mmHg; $P < 0.05$) strains. Systolic pressure in MFS rats (171 ± 6 mmHg) was not significantly different from the other strains (Table 1). There was no significant difference between the four groups in diastolic pressure, which averaged 140 ± 3 mmHg.

Intraventricular injections of neostigmine (5 μg) evoked a significant rise in arterial blood pressure in three of the four strains; MFS, CS, and MLE. The mean systolic pressure responses were, respectively, 55 ± 7, 61 ± 5 and 71 ± 8 mmHg (Table 1). These responses usually occurred within 25-90 s of injection.

In contrast to these effects, neostigmine (5 μg) evoked only threshold cardiovascular responses in CFE rats. Doses of 20-30 μg were required in these animals to evoke a rise in blood pressure equivalent to that observed in the other strains (Table 1). Furthermore, the latency to onset of the hypertensive effects was nearly 3 min; considerably longer than observed in the MS, MLE or CS strains.

As shown in Table 1, prior (2 h) intraventricular injection of hemicholinium-3 (HC-3) in a dose of 0.4 μg, reduced the pressor response to neostigmine in MFS and MLE rats to less than 50% of control. In contrast, the response in the CS strain was only slightly inhibited (89% of control) and the CFE strain was completely unaffected.

When the dose of HC-3 was increased to 40 μg there was only a slight additional degree of inhibition in the MS and MLE strains, but the pressor response in the CS animals was reduced to 53% of control. Notably, however, the response to neostigmine in the CFE strain was still not significantly reduced by this dose of HC-3.

Discussion In a recent communication (Brezenoff, 1973b) it was reported that reduction of brain acetylcholine with HC-3 failed to modify the pressor response to i.c.v. injection of neostigmine, suggesting a direct central action of neostigmine. The present study, however, demonstrates strain differences in the degree to which HC-3 inhibits this hypertensive response.

In two strains, MFS and MLE, pretreatment with 0.4 μg of HC-3 reduced the pressor response by approximately 50%. This inhibition was only slightly augmented by a 100-fold increase in the dose of HC-3, suggesting that nearly maximal inhibition is achieved with the lower dose. Preliminary experiments have shown that in the MFS strain, 0.4 μg of HC-3 produces nearly maximal depletion of brain acetylcholine (approximately 80%) 2 h after intraventricular injection (Brezenoff & Rusin, 1974). Thus approximately half of the pressor response to neostigmine in the MFS and MLE strains appears to be mediated through brain acetylcholine, while the remainder may result from direct central actions of the drug.

The CS strain was more resistant to the inhibitory effects of the low dose of HC-3, but with a 100-fold increase in dose (40 μg) the pressor response was reduced approximately 50%. In contrast, 40 μg of HC-3 did not reduce the pressor response in the CFE strain. Thus, depending upon the strain examined, different interpretations could be made as to the role of brain acetylcholine in the pressor response to intraventricularly injected neostigmine.

It appears likely from the data that neostigmine can evoke a rise in arterial blood pressure by either a direct action on central neurones, or by inhibition of brain acetylcholinesterase and subsequent increase in local concentrations of acetylcholine. The present study suggests that the relative contribution of each of these actions may vary, depending upon the strain of rat examined.

Table 1 Effects on blood pressure of intraventricularly injected neostigmine in control rats of different strains

	Strain			
Cardiovascular parameter	*MFS*	*MLE*	*CS*	*CFE*
Neostigmine dose (μg)	5	5	5	30
Predrug systolic pressure (mmHg)	171 ± 6*	158 ± 4	164 ± 3	176 ± 4
Neostigmine pressor response (mmHg)	55 ± 7	71 ± 8	61 ± 5	65 ± 7
% of control pressor response in HC-3-treated rats 0.4 μg	48 ± 9	47 ± 8	89 ± 3	100 ± 10
40.0 μg	41 ± 12	34 ± 4	53 ± 4	95 ± 9

* Each value represents the mean of 4-8 experiments.

The effects of HC-3 on the pressor response were nearly identical in the two different strains (MLE and MFS) obtained from the same breeder, while considerable variability occurred between Sprague-Dawley derived rats obtained from different breeders. This suggests that environmental conditions may play a significant role in the varied drug responsiveness observed in this study. Environmental differences have been shown to alter central cholinesterase activity (Rosenzweig, Bennett & Diamond, 1967).

While we cannot as yet explain the cause of the strain difference, it is hoped that the present communication will prevent possible discrepancies from appearing in the literature based on the previous findings in a strain of rats apparently resistant to the inhibitory effects of HC-3 on the central response to neostigmine.

References

BREZENOFF, H.E. (1973a). Centrally induced pressor responses to intravenous and intraventricular physostigmine evoked via different pathways. *European J. Pharmac.*, **23**, 290-292.

BREZENOFF, H.E. (1973b). The role of brain acetylcholine in the pressor response to centrally injected neostigmine. *Br. J. Pharmac.*, **49**, 557-559.

BREZENOFF, H.E. & RUSIN, J. (1974). Brain acetylcholine as a mediator of the pressor response to physostigmine in the rat. *Fedn. Proc.*, **33**, 506.

DIRNHUBER, P. & CULLUMBINE, H. (1955). Effect of anticholinesterase agents on rats blood pressure. *Br. J. Pharmac. Chemother.*, **10**, 12-15.

ROSENZWEIG, M.R., BENNETT, E.L. & DIAMOND, M.C. (1967). Effects of differential environments on brain anatomy and brain chemistry. *Proc. Amer. Psychopath. Assoc.*, **56**, 45-56.

VARAGIĆ, V. (1955). The action of eserine on the blood pressure of the rat. *Br. J. Pharmac. Chemother.*, **10**, 349-353.

VARAGIĆ, V. & VOJVADIĆ, N. (1962). Effect of guanethidine, hemicholinium and mebutamate in the hypertensive response to eserine and catecholamines. *Br. J. Pharmac. Chemother.*, **19**, 451-457.

(Received April 2, 1974)