The influence of 6-OHDA on hypothermia produced by intrahypothalamically-injected carbachol in the pigeon

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Summary. The involvement of the noradrenergic system in hypothermia induced by intrahypothalamically-injected carbachol (CCh) was studied by depleting hypothalamic noradrenaline (NA) with the catecholamine neurotoxin 6-hydroxydopamine (6-OHDA) and repeating the CCh injections after 6-OHDA treatment. The results suggest that noradrenergic neurons may be involved in hypothermia produced by CCh in the pigeon.

It is suggested that acetylcholine (ACh) in the noradrenergic nerve endings may cause the liberation of NA[1]. Burn proposed that the release of NA from the sympathetic fibres is preceded by the release of ACh which in turn releases NA from the vesicles[2]. It is suggested, too, that endogenous NA might indirectly be involved in a slight decrease of body temperature (T_b) induced by intrahypothalamic injection of ACh + physostigmine in the pigeon[3]. It has been shown that under thermoneutral conditions the intrahypothalamic injection of NA[4] and cholinomimetics (e.g. CCh[5]) induces hypothermia in the pigeon. On this basis the interaction between the central noradrenergic and cholinergic systems in temperature regulation in the pigeon was studied. The acute effects of 6-OHDA on body temperature were also recorded.

Materials and methods. Adult pigeons *(Columbia livia)* of either sex, weighing 270–340 g, were used in this study. The care of the animals has been described earlier[5,6]. A guide cannula was implanted stereotaxically[7,8] into the hypothalamus under sodium pentobarbital anesthesia. The coordinates used were A 8.0 mm, L 1.5–2.0 mm and 9.0–9.5 mm below the skull surface. Microinjections (1 µl) into the hypothalamus were made according to the following schedule: first CCh (1.5 µg), after 5 or 6 days 6-OHDA (8 µg) and finally 3 days after 6-OHDA injection CCh (1.5 µg) once again. Carbamylcholine chloride (Carbachol, CCh, Sigma) was dissolved in distilled water and 6-hydroxydopamine hydrobromide (6-OHDA, Sigma) in 0.85% saline containing ascorbate (1 mg/ml) as an antioxidant. Measurement procedures used in this study have been described earlier[5]. The measurements were carried out with unanaesthetized pigeons at 20 °C.

Results and discussion. Injection of CCh produced hypothermia in all pigeons. The difference in the responses before and after 6-OHDA treatment was not significant (figure 1, A).

2 kinds of acute effects of 6-OHDA were noted. In 4 pigeons (Nos 160, 161, 169, 170) the injection intensified shivering and increased T_b (peak effect 0.4 ± 0.13 °C, $\bar{x} \pm$ S.E., reached in 15 min) and in 2 birds (Nos 157, 159) it inhibited shivering for about 4 min and evoked a temperature fall (maximally 0.3 °C) within 6 min after injection.

In the pigeons in which 6-OHDA caused hypothermia, CCh before 6-OHDA treatment induced cessation of shivering for 70 min and lowered T_b on the average by 2.6 °C. Injection of CCh 3 days after 6-OHDA again produced hypothermia, though it was much weaker. Shivering was absent only 19 min and T_b decreased by 0.5 °C (figure 1, B).

In the pigeons in which 6-OHDA raised T_b, CCh before 6-OHDA induced cessation of shivering for 19 ± 4 min and T_b decreased 1.5 ± 0.38 °C. In this group 6-OHDA treatment did not significantly affect the CCh-produced hypothermia, although the responses after treatment were more pronounced than before. Shivering was absent 24 ± 6 min and T_b was lowered by 1.8 ± 0.46 °C (figure 1, C).

The injection sites located in the anterior hypothalamus are illustrated in figure 2.

The results with CCh confirm our findings of the hypothermic effect of cholinomimetics in the anterior hypothalamus of the pigeon[5]. It has been reported that in the rat the most effective dose of 6-OHDA for specific depletion of NA in the catecholamine-containing terminals in the brain is 2 µl of a 8-µg/µl solution[9]. Smaller or larger doses induced only nonspecific damage. Accordingly 8 µg of 6-OHDA was used in this work.

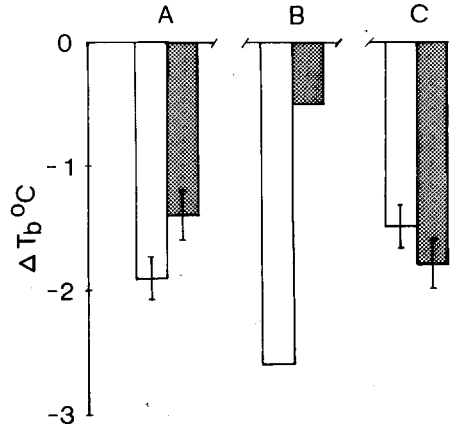

Fig. 1. Records of mean maximum changes in body temperature (ΔT_b) to CCh in all pigeons used at T_a 20 °C (*A*). *B* illustrates records in the pigeons in which 6-OHDA induced hypothermia. *C* presents results in the pigeons in which 6-OHDA increased temperature. Bars indicate SE of the mean. Open columns; responses to CCh before 6-OHDA injection. Filled columns; responses to CCh 3 days after 6-OHDA injection.

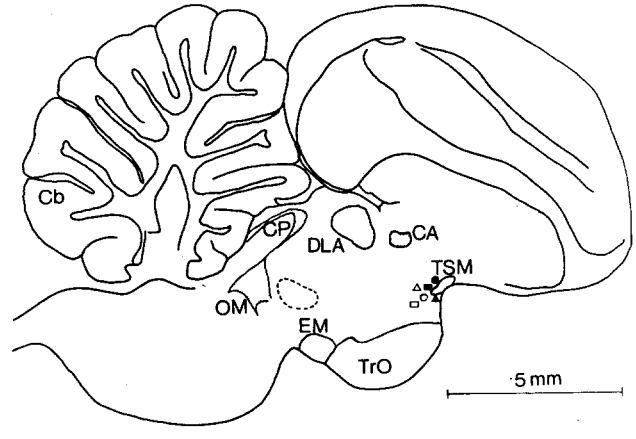

Fig. 2. The sagittal section of the pigeon brain 1.5–2.0 mm lateral to the midline indicating the locations of the cannula tips in 6 pigeons (□ 157, ○ 159, △ 160, ■ 161, ● 169, ▲ 170). Abbreviations: CA, commissura anterior; Cb, cerebellum; CP, commissura posterior; DLA, nucleus dorsolateralis anterior thalami; EM, nucleus ectomamillaris; OM, tractus occipitomesencephalicus; TrO, tractus opticus; TSM, tractus septomesencephalicus.

The acute effect of 6-OHDA in the present study was either an increase or a fall in T_b. The hypothermic effect of 6-OHDA might result from the release of endogenous NA[10], which in turn could activate pathways driving inhibition of heat production. The lowering effect of 6-OHDA on T_b has been widely demonstrated in mammals [11-13]. The hypothermic effect of intrahypothalamic injection of dopamine (DA) on T_b of the pigeon seems to be rather slight[3]. Since the highest concentration of DA is found outside the hypothalamus[14,15] interaction between dopaminergic and cholinergic system seems unlikely. Furthermore the effects obtained with 6-OHDA are not necessarily a result from its effect on dopaminergic neurons.

The slight increase in T_b, when the injection site was close to the tractus septomesencephalicus, points to a transient, probably unspecific activation of pathways driving thermogenesis. This indicates that 6-OHDA did not reach the catecholamine terminals, hence NA was not released and no hypothermia ensued.

In discussing the possibility of the interaction between the noradrenergic and cholinergic systems, an assumption is made that stimulation of either of these systems simultaneously with the other adds to hypothermia induced by one system alone. Since near the tractus opticus the hypothermic response to CCh was markedly attenuated in birds in which 6-OHDA induced a decline of T_b, we might reason that due to the degeneration of NA terminals this system could not contribute to CCh hypothermia. This also indicates that the noradrenergic system is involved in cholinergic hypothermia via the release of NA.

In conclusion, the results of the present work suggest that in a circumscribed area in the pigeon hypothalamus the noradrenergic neurons may be involved in hypothermia induced by injection of CCh.

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Ultrastructural preservation of human atrial intrinsic innervation after the cold ischemic anoxic asystole during cardiac surgery[1]

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Summary. The method of induced cold ischemic anoxic asystole used in the course of cardiac surgery for protection of the myocardium also preserves well the inbuilt intrinsic nervous apparatus of the heart, including the small dense-cored vesicles of the adrenergic nerve terminals. However, if the protective procedure fails, the resulting damage to the myocardial cells is accompanied by severe destruction of the neural elements.

Protection of the myocardium from ischemic anoxic injury during open-heart surgery is of crucial importance in order to ensure adequate cardiac function after the corrective procedure. Various techniques have been widely used for myocardial preservation. The efficacy of these techniques has been studied also with the electron microscope[2-7]. Attempts have been made to define criteria of irreversible damage[6], and the ultrastructural picture of the 'stone heart' (extreme irreversible ischemic contracture of the left ventricle following extended normothermic ischemic arrest) has been described also in humans[8]. Until now, the main interest has been focused on the myocardial cell, while very little is known of possible injury to the inbuilt intrinsic nervous apparatus of the heart. The present study was undertaken partly to fill this gap in our knowledge.

Patients and methods. Right atrial myocardial biopsies were excised in the course of prosthetic aortic valve replacement operation from 8 consecutive patients a) before starting the extra-corporeal circulation, b) after the cold ischemic anoxic asystole (aortic cross-clamping (50–70 min) during general hypothermia (30 °C) associated with local cardiac cooling with +4 °C saline solution) and subsequent coronary reprefusion (20–30 min, until decannulation). 2 specimens were excised from each patient. The 1st specimen (which served as the control) was excised from the right auricular appendage at the insertion of the venous perfusion cannula. The 2nd specimen was excised from the root of the right auricular appendage, below the purse-string suture, at decannulation. Thin myocardial tissue strips were immediately fixed by immersion in 5% glutaraldehyde (in 0.1 M phosphate buffer, pH 7.2) at 0 °C for 5 h, postfixed in 2% osmium tetroxide for 1 h, dehydrated in graded series of aethyl alcohol, and finally embedded, through propylene oxide and a mixture of equal amounts of propylene oxide and epoxy resin, in an epon-araldite mixture. Ultrathin sections exhibiting silver to gold interference colours were stained with saturated uranyl acetate and 2.5% lead citrate. The sections were viewed and photographed with a Philips electron microscope EM 300, operated at 60 kV.

Observations. In most cases, the ultrastructure of the nerve