The effects of brain monoamine depletion on p-chlorophenyl-alanine-induced hypothermia[1]

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Summary. I.p. administration of p-chlorophenylalanine produced a fall in rectal temperature in rats. The hypothermia was attenuated after pretreatment of the animals with 5,6-dihydroxytryptamine, but was unaffected after pretreatment of the animals with 6-hydroxydopamine.

It is well known that 5-hydroxytryptamine (serotonin; 5-HT) is not evenly distributed throughout the brain and that the highest concentration of this amine occurs predominantly in the hypothalamus[2]. Moreover, there is growing evidence that inputs project from the peripheral thermoreceptors to synaptic relays in the 5-HT cell bodies in the midbrain, and thence to the hypothalamus via the serotoninergic pathways[3]. It is inviting, therefore, to think that drugs interfering with the metabolic pathways of 5-HT, might exert an influence on temperature regulation. Indeed, the majority of studies which have examined body temperatures after p-chlorophenylalanine (pCPA) treatment reported hypothermia lasting for several h, in spite of the fact that maximal depletions of brain 5-HT were not achieved during this period[4,5,6]. The aim of the present study was to assess further the possible relationship between brain monoamines and acute hypothermia induced by pCPA in unanesthetized rats at room temperature (22 °C).

Material and methods. 48 males of Sprague-Dawley strains, ranging between 250 and 300 g at the time of experimentation, served as subjects. The animals being housed individually in wire-mesh cages in a room at 25±1.0 °C with a natural light-dark cycle were given free access to tap water and granular chicken feed. 3 groups of animals were used in the present experiments: a) sham-treated control rats, b) rats with intraventricular administration of an aliquot of 100 µl containing 100 µg of 5,6-dihydroxytryptamine (5,6-DHT, Sigma), resulting in long-lasting depletion of 5-HT in the CNS[7-9], and c) rats with intraventricular administration of an aliquot of 100 µl containing 200 µg of 6-hydroxydopamine (6-OHDA, Sigma), resulting in long-lasting depletion of catecholamine (CA) in the CNS[7,10,11]. The animals treated with 5,6-DHT were studied between 2 and 7 days after the injections were made, while the animals treated with 6-OHDA were studied between 1 and 2 weeks after the injections were made. Each of the above rats had been implanted with an intraventricular cannula under general anesthesia (sodium pentobarbital, 6 mg/100 g, i.p.). The cannulae were located in the lateral ventricle; the stereotaxic coordinates were 3 mm lateral and 7 mm caudal to the Bregma. During the surgery, the correct positioning of each guide tube was verified by the rapid flow of saline into the ventricle under gravity using an injecting needle. Instead of the drugs, the sham-treated controls were injected with 0.9% of saline. The thermoregulatory responses of these groups of animals, 2 weeks after recovery from the operation, to pCPA (Pfizer Inc., 300 mg/kg, i.p.) were observed. Metabolic rate (M), respiratory evaporative heat loss (E_{res}), rectal (T_r) and skin temperatures were measured or calculated[12].

Results and discussion. Figure 1 summarizes the temperature responses of both groups of 5,6-DHT-treated and 6-OHDA-treated rats to an i.p. dose of 300 mg/kg of pCPA and a comparison of these responses to those of untreated controls. In control group, i.p. treatment of pCPA produced a hypothermia of 2.2±0.25 °C; the hypothermia was brought about by an increase in heat loss, both an increase in E_{res} and in the skin temperature of the feet (T_f). During the 90-min period of hypothermia genesis, both the maximal E_{res} and T_f values were 0.4 W/kg and 6.0 °C respectively higher than the values measured before the pCPA treatment (figure 2). There were no alterations in heat production. Figure 1 also shows that pCPA-induced hypothermia was attenuated after pretreatment of the animals with 5,6-DHT, but was unaffected after pretreatment of the animals with 6-OHDA. In addition, brains analyzed for 5-HT content were obtained from 5 saline-treated rats, from 5 rats 3 days after injection with 5,6-DHT, and from 5 rats 1 week after injection with 6-OHDA. The methods used for the determination of 5-HT are based on Atack and Lindqvist[13]. Biochemical data revealed that animals treated with 5,6-DHT showed a greater than 60% decrease in brain 5-

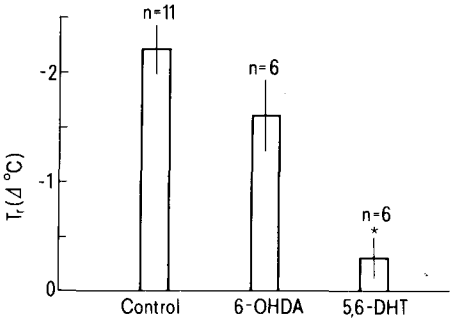

Fig. 1. Mean reduction in rectal temperature (Tr) of control, 5,6-dihydroxytryptamine-treated (5,6-DHT) and 6-hydrodopamine-treated (6-OHDA) rats in response to i.p. administration of p-chlorophenylalanine, 300 mg/kg, at ambient temperature of 22 °C. The vertical bars represent ±SEM. n, numbers of rats tested. *: significantly different from control value, $p < 0.05$ (Student's t-test).

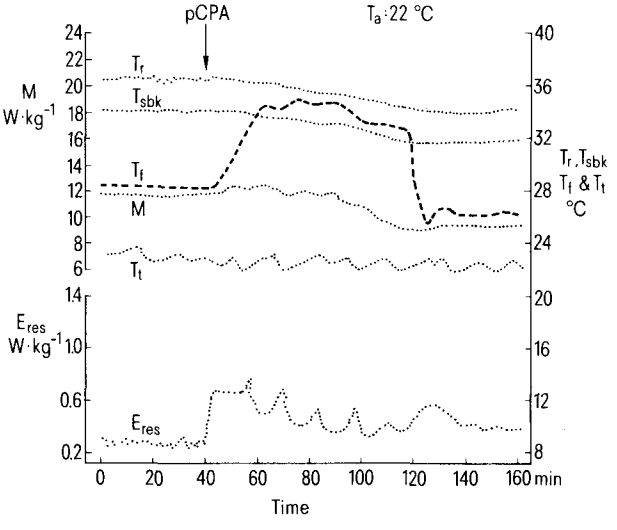

Fig. 2. Record of rectal temperature (T_r), metabolic rate (M), respiratory evaporative heat loss (E_{res}), back (T_{sbk}), feet (T_f) and tail (T_t) skin temperatures from a control rat at an ambient temperature (T_a) of 22 °C. At the arrow, injection into the peritoneal cavity of 300 mg/kg of p-chlorophenylalanine (pCPA).

HT content, while animals treated with 6-OHDA showed an insignificant change in brain 5-HT content. Thus the data suggest that pCPA may act through the serotoninergic systems to exert its hypothermic action.

In fact, there is growing evidence suggesting a hypothermic role for brain 5-HT in rats. For example, it has been found that intraventricular administration of 5-HT led to hypothermia in rats[14,15]. Moreover, in this laboratory, we found that elevating 5-HT content in brain with 5-hydroxytryptophan reduced rectal temperature in rats (Lin et al., unpublished data) after peripheral decarboxylase inhibition with R04-4602 at ambient temperatures of both 8 and 22 °C. Also, elevating 5-HT concentration in 5-HT receptor sites or increasing functional 5-HT in brain with inhibitors of 5-HT re-uptake such as Lilly 110140 and chlorimipramine reduced rectal temperature in rats at room temperature and below (Lin et al., unpublished data). This raises the possibility that pCPA may act through the enhanced release of brain 5-HT to induced hypothermia. However, it must be acknowledged that pCPA is used as a 5-HT synthesis inhibitor rather than a 5-HT precursor[5]. It is not known whether the immediate inhibition of 5-HT synthesis after pCPA treatment would induce an enhanced release of 5-HT into synaptic clefts and result in a transient acute hypohtermia. The possible feedback mechanism needs further verification.

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2 A.H. Amin, T.B.B. Crawford and J.H. Gaddum, J. Physiol. (Lond.) *126*, 596 (1954).
3 R.F. Hellon, Pharmac. Rev. *26*, 289 (1975).
4 N.J. Giarman, C. Tanaka, J. Mooney and E. Atkins, Adv. Pharmac. *6A*, 307 (1968).
5 K. Koe and A. Weissman, J. Pharmac. exp. Ther. *154*, 499 (1966).
6 A.R. Somerville and B.A. Whittle, Br. J. Pharmac. *31*, 120 (1967).
7 M.T. Lin, W.Y. Chia, C.T. Tsai and T.H. Yin, Experientia *34*, 756 (1978).
8 H.G. Baumgarten, H.G., A. Bjorklund, A. Holstein and A. Nobin, Z. Zellforsch. *129*, 256 (1972).
9 A. Bjorklund, A. Nobin and U. Stenevi, Brain Res. *53*, 117 (1973).
10 J.G.V. Zoeren and E.M. Stricker, Am. J. Physiol. *230*, 932 (1976).
11 M.T. Lin and J.T. Stitt, Physiologist *19*, 271 (1976).
12 M.T. Lin, J. Pharmac. exp. Ther. *204*, 39 (1978).
13 C. Atack and M. Lindqvist, Naunyn-Schmiedebergs Arch. Pharmac. *279*, 267 (1973).
14 W. Feldberg and V.J. Lotti, Br. J. Pharmac. *31*, 152 (1967).
15 R.D. Myers and T.L. Yaksh, Physiol. Behav. *3*, 917 (1968).

Permeability coefficients of the egg-case membrane of *Scyliorhinus canicula* L.

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Summary. The cleidoic egg-case of the dogfish appears to have a highly porous and permeable outer membrane, the pore radius being computed to be 13.6 Å. It does not present any physiological barrier to small molecules and therefore constitutes an open ionic and osmotic system for the embryo. Being a porous protein membrane it may be of value as a model for molecular transport mechanisms.

The oviparous dogfish, *Scyliorhinus canicula* L. lays egg-cases throughout the year and for the first 3 months of their development, until the embryo is 3 cm long, the eggs are cleidoic[1]. Because of the osmotic importance of urea to Elasmobranchs, both Wood[2] and Baldwin[3] have suggested that the egg-case is impermeant to this solute, it being considered necessary to retain urea within the case for the embryo's survival. The urea molecule has a radius of approximately 2.3 Å which is much the same size as the water molecule. Does this imply that the egg-case is impermeant to water as well? The shark gill has been shown[4] to be relatively impermeant to urea but freely permeable to water. Can the mechanism of urea retention in the living gill be similar to that in the dead collagenous egg-case? In this report, the permeability of the egg-case to water and some selected solutes is established.

3 coefficients of permeability categorise a membrane[5], L_p, the hydraulic coefficient, ω, the diffusional coefficient, and σ_s, the reflection coefficient. ω is related to the conventional permeability coefficient P, by $P=\omega RT$ (R and T being the gas constant and absolute temperature respectively). The volume flux, J_v, brought about by an osmotic gradient, $\Delta\pi_s$ is given by $J_v=-L_p\sigma_s\Delta\pi_s$[6], the negative sign being convention. The reflection coefficient is a measure of the 'leakiness' of the membrane to the solute. When $\sigma_s=1$, s is impermeant and when $\sigma_s=0$, s is permeant.

Methods. Areas of washed membranes were clamped between 2 glass reservoirs containing the experimental solutions. The apparatus was shaken horizontally in a water bath at 293 °K. To measure L_p, a solution of an impermeant solute, polyvinylpyrrolidone PVP ($\Delta\pi_s=21.6$ at) was placed in 1 reservoir with a weighed amount of distilled water in the opposing one. Gravimetrically, J_v was measured and L_p calculated. ω was determined for water with ^3H_2O (0.2 μCi ml^{-1}) in 1 compartment and assaying its appearance in the water of the opposing one. This experiment returned a value for ω_T (T for tritiated water) calculated from the modified Fick equation:

$$P_T=\omega_T RT=\frac{Q}{\Delta C\cdot A\cdot t}$$

where Q is the total amount of label moved in time t across an area A cm^2 under an isotope gradient ΔC. P_s or ω_s for various labelled solutes may similarly be determined. With the measured value of L_p calculated using PVP, the J_v produced by a permeant solute was used to measure the relative σ_s for that particular solute. Corrections for the unstirred layers[7] of water were unnecessary for a membrane this thick (0.4 mm).

The values for the permeability coefficients for the egg-case are shown in table 1. Whether or not there is an osmotic gradient across the membrane, the permeability coefficient, P_T, for water movement is high compared to the J_v values and is fairly constant indicating that the major contribution to water movement is purely diffusional and not bulk flow. By converting the units of ω_T to those of L_p, by multiplying by the molal volume of water