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EFFECT OF INTRAHYPOTHALAMIC PHENTOLAMINE ON HYPOTHERMIA PRODUCED BY PERIPHERAL NORADRENALINE IN THE PIGEON

R. HISSA & A. PYÖRNILÄ

Zoophysiological Laboratory, Department of Zoology, University of Oulu, 90100 Oulu 10, Finland

1 The effect of intrahypothalamic phentolamine (1 and 2 μl of a 10 μg/μl solution) on hypothermia induced by intramuscular injection of noradrenaline (NA, 2 mg/kg) at different times (2, 10, 20 min) after phentolamine was investigated in pigeons.

2 Administration of phentolamine shortly before NA was shown to attenuate hypothermic responses to NA.

3 It is suggested that the attenuation is due to blockade of central hypothermic effects of intramuscular NA.

4 The mapping of injection sites in the brains was carried out.

Introduction

Although catecholamines including noradrenaline (NA) are generally believed to be incapable of penetrating the blood-brain barrier of adult animals in more than minute amounts (Whitby, Axelrod & Weil-Malherbe, 1961; Oldendorf, 1971; 1974), these amines, even when peripherally applied, may have central thermoregulatory effects (Allen & Marley, 1967; Feldberg & Lotti, 1967). In birds, peripheral (Allen & Marley, 1967; Allen, Garg & Marley, 1969; 1970; Hissa, Pyörnilä, & Saarela, 1975a; Hissa, Saarela & Pyörnilä, 1975c), intracerebroventricular (Grunden & Marley, 1970; Chawla, Johri, Saxena & Singhal, 1974) and intrahypothalamic (Marley & Stephenson, 1968; 1969; 1975; Hissa & Rautenberg, 1974; 1975) injections of catecholamines at or below thermoneutrality have been shown to induce hypothermia. Chawla *et al.* (1974) and Hissa & Rautenberg (1975) demonstrated that the hypothermic effect of centrally injected NA was diminished or totally prevented by an intracerebral injection of phenoxybenzamine or phentolamine. Based on these findings it seemed logical to ask whether an intrahypothalamic injection of an α-adrenoceptor blocking agent might also affect the hypothermia induced by a peripheral injection of NA. This has been tested in conscious pigeons.

Methods

Adult pigeons (*Columba livia*) of either sex (weight 300–350 g) were used. The birds were trapped at Turku, SW-Finland in October-November, and the experiments carried out in January-June. The care of birds has been described earlier (Pyörnilä, Hissa & Saarela, 1976).

Stereotactic implantation of a guide cannula into the hypothalamus was performed as described by Hissa & Rautenberg (1974). The birds were allowed a week for postoperative recovery and were then tested with an intrahypothalamic injection of NA (10 μg/μl) at an ambient temperature (*t*a) of 6°C. Pigeons that responded with hypothermia (see Hissa & Rautenberg, 1974) were used further. In experiments at *t*a 6°C, phentolamine (10 μg/μl or 20 μg/2 μl) was injected into the hypothalamus followed by NA (2 mg/kg) intramuscularly at different times (2, 10, 20 min) after phentolamine.

The method for measuring O_2 consumption (Beckman E2 Analyzer) was that described by Hissa & Palokangas (1970). Also previously described were the techniques for intrahypothalamic and peripheral injections (Hissa & Rautenberg, 1974; Hissa *et al.*, 1975c; Pyörnilä *et al.*, 1976) and the methods for measuring body (*t*b) and foot temperatures (*t*f) (Ellab Z8 recorder) and shivering (breast muscle electrodes and Schwarzer Varioscript V822 polygraph, see Saarela, Mattila, Hissa & Hohtola, 1976). At the end of the experiments the birds were killed and the brains fixed in 10% formalin. Paraffin sections (15–20 μm) were stained with cresylviolet and examined for the location of the injection cannula.

An experiment was not begun until stable baselines for *t*b and O_2 consumption were obtained (usually within 2 hours). In each experiment the mean *t*b and

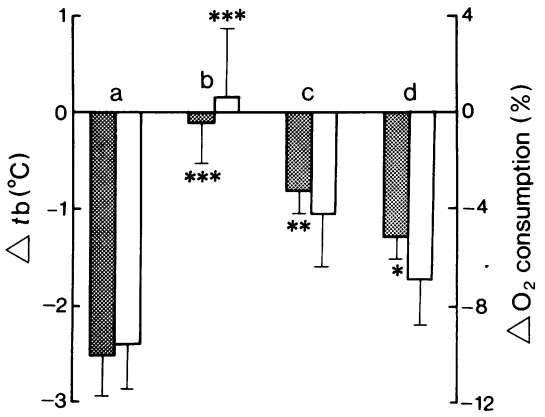

Figure 1 Mean maximum change in body temperature (Δtb, stippled columns) and O_2 consumption (open columns) in response to noradrenaline (NA, 2 mg/kg, i.m.) alone and after pre-injection of phentolamine into the hypothalamus. (a) NA without phentolamine (10 experiments on 7 pigeons); (b) NA 2 min after phentolamine, 20 µg/2 µl (16 experiments on 7 pigeons); (c) NA 10 min after phentolamine, 20 µg/2 µl (13 experiments on 6 pigeons); (d) NA 20 min after phentolamine, 10 µg/µl (11 experiments on 6 pigeons). Vertical bars represent s.e. means. Asterisks refer to comparison between control (a columns) and phentolamine-treated birds. $^{***}P<0.001$; $^{**}P<0.01$; $^{*}P<0.05$ (Student's *t* test).

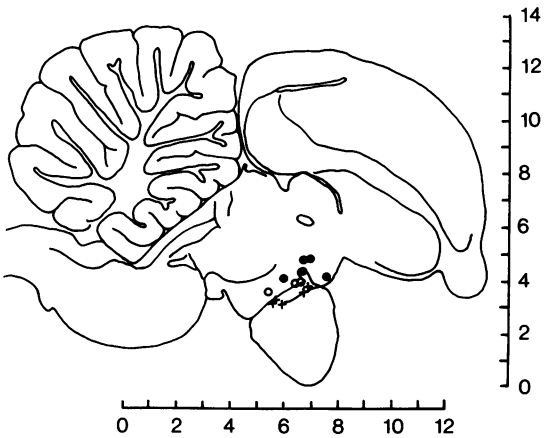

Figure 2 Injection sites of phentolamine in the pigeon brain shown at 1.0–2.0 mm lateral to the midline. (●) Prevention or attenuation of noradrenaline (NA, i.m.)-induced hypothermia; (○) no effect on hypothermia; (+) death after NA. The drawing is based on the Karten & Hodos (1967) atlas with the modification of mm scale to fit the smaller brain size of the birds used in the present work.

O_2 consumption (readings at 4 min intervals) during 20 min preceding phentolamine or NA injection were calculated, and individual measurements throughout an experiment were plotted as the difference from this mean. Baseline values of O_2 consumption ranged between 2.01 and 3.10 ml min^{-1} 100 g^{-1}, and of *tb* between 37.9 and 41.1°C. *tf* varied between ca. 20° and 30°C.

Drugs used were: (–)-noradrenaline (L-arterenol bitartrate, Sigma) dissolved in 0.85% saline and phentolamine (Regitine, Ciba). The dose of NA (2 mg/kg) refers to base.

Results

Figure 1 summarizes the results obtained. Measurements were made on 8 birds but, due to dislodgement of the cannula in a few birds, not all the experiments could be accomplished according to the planned schedule.

When injected alone, NA (2 mg/kg i.m.) induced hypothermia and a fall in O_2 consumption, responses very similar to those observed earlier (Hissa *et al.*, 1975c). The maximum mean falls of *tb* and O_2 consumption were 2.6°C and ca. 9% (10 experiments in 7 pigeons, Figure 1a), respectively.

Following injection of phentolamine (20 µg/2 µl) into the hypothalamus and NA (2 mg/kg i.m.) 2 min later, irregular responses were seen. In 3 pigeons (7 experiments) a slight rise of *tb* and in 4 pigeons (9 experiments) a small fall was noted. In each case, a parallel change in O_2 consumption was seen, suggesting that the change in *tb* was related to metabolic heat production. The mean effect for the group was a fall of 0.1°C in *tb* and a rise of about 0.6% in O_2 consumption (Figure 1b).

The injection of NA (2 mg/kg i.m.) 10 min after intrahypothalamic injection of phentolamine (20 µg/2 µl) resulted in falls of *tb* and O_2 consumption in 11 experiments on 6 pigeons. A fall was noted in one other experiment on a pigeon which also responded with slight increases of *tb* and O_2 consumption in two experiments. When 10 µg/µl of phentolamine was injected into the hypothalamus and NA (2 mg/kg i.m.) injected 20 min later, hypothermia developed in 5 pigeons (10 experiments) and in one bird only (one experiment) a slight rise in *tb* and O_2 consumption was seen. The maximum mean falls over the 2–2.5 h experimental period for these two groups were 0.8°C and 1.3°C in *tb* and ca. 4% and 7% in O_2 consumption, respectively (Figure 1 c and d). The injection of saline (either 1 µl or 2 µl) into the hypothalamus did not affect the hypothermic responses elicited by intramuscular NA.

There were great individual variations among the pigeons with respect to shivering. After NA alone, shivering was absent for 15 min on average.

Generally, phentolamine pretreatment reduced this period and following 20 μg phentolamine 2 min before NA, shivering was maintained throughout, although slightly depressed in intensity.

Although the changes of *tf* were variable, intramuscular NA usually caused a fall of 4–8°C if the initial level was high (28–32°C) at the time of injection. When initially low (below and around 20°C), a transient rise (2–8°C) of *tf* was often seen.

Phentolamine itself seemed to have only a small effect on *tb*. The dose of 10 μg/μl caused a delayed slow rise (0.6 ± 0.07°C, $n=5$), and the maximum was reached at a latency of 60 to 80 min after injection. On the other hand, the dose of 20 μg/2 μl lowered *tb*, the fall averaging 0.6 ± 0.28°C ($n=5$) and the maximum being reached 30–95 min after administration. No noteworthy effect on shivering or O_2 consumption was seen.

Five pigeons (not included in the results presented above) died in the first experiment (20 μg/2 μl phentolamine injected 2 min before NA). The birds seemed to have difficulties in breathing and death occurred within 10–20 min after NA administration. At that time the body temperature exhibited no abnormal deviations from those usually observed in connection with NA injections. The injection sites in these birds are included in the mapping of cannula locations shown in Figure 2.

Discussion

Intrahypothalamically applied phentolamine caused an attenuation of the hypothermic effects of the intramuscular injection of NA. It has previously been shown that pretreatment with phentolamine prevents hypothermic effects of NA when both drugs are applied either into the hypothalamus (Hissa & Rautenberg, 1974) or intramuscularly (Hissa *et al.*, 1975c). Since different routes for phentolamine and NA were used in the present experiments, the basic question to be answered is whether the two drugs interact at the same site to cause the observed effects or, if different sites in both periphery and CNS are involved, how this could account for the results.

No specific data about the fate of intramuscular NA in pigeons can be given but, provided that NA binding in avian tissues is similar to that in mammals (Whitby *et al.*, 1961; Cohen & Rodriguez-Farre, 1975), a substantial proportion of the NA would be inactivated by binding to plasma proteins and tissue parenchyma. Among the peripheral effects of NA, a fall in *tf* presumably due to the constrictor effects on blood vessels in the legs was observed. Similarly, NA might affect shivering by reducing blood flow through the breast muscle. The possibility of a metabolic stimulatory effect of NA on the pigeon has been discussed previously (Hissa, Rantala & Jeronen, 1975b; Hissa *et al.*, 1975a, c; Pyörnilä *et al.*, 1976). Nevertheless, the primary effect of intramuscular NA is considered to be central and inhibitory on heat production at low *ta* (Hissa *et al.*, 1975a, c).

Injection of propranolol (i.m.) was shown not to affect the hypothermic effect of intramuscular NA at *ta* 6°C (Hissa *et al.*, 1975c). Assuming that propranolol enters the brain (see Masuoka & Hansson, 1967; Laverty & Taylor, 1968) and that the NA-induced hypothermia is mainly of central origin, the hypothermic response cannot then be attributed to β-adrenoceptor activation in the brain. In fact, mediation by α-adrenoceptors has been shown (Hissa *et al.*, 1975c).

Intrahypothalamic injection of 1 μg NA produced hypothermia in the pigeon and was blocked by a small amount of phentolamine (1–5 μg/μl) (Hissa & Rautenberg, 1975). Because of the small doses needed, it would appear that reversal of the hypothermic effect of intramuscular NA (2 mg/kg) by pretreatment with phentolamine (10 mg/kg i.m.) (Hissa *et al.*, 1975c) might also be due to a central effect, despite a rather poor passage of phentolamine into the brain (see Andén & Strömbom, 1974). On this basis, it was not surprising that the hypothermic effect of intramuscular NA was attenuated by phentolamine after direct blockade of α-adrenoceptors in the hypothalamus. On the whole, this evidence suggests that central stimulation of α-adrenoceptors is involved in the mediation of hypothermia induced by NA injected either centrally or peripherally into the pigeon at *ta* 6°C. Recently, however, Nisticò, Marley & Preziosi (1976) have demonstrated that in adult fowls antagonists at β-adrenoceptors may also prevent the hypothermic effects of catecholamines infused into the hypothalamus.

Intrahypothalamic phentolamine itself caused only a small and slow change in *tb*. On the assumption that NA is released continuously in the hypothalamus to control *tb* (Feldberg & Saxena, 1971), this might be explained by a rather low rate of release of NA at the time of injection (after ca. 2 h exposure of birds to *ta* 6°C). In view of the hypothermic effects of NA, this would not appear unreasonable. Chawla *et al.*, (1974) demonstrated a small and slowly developing hyperthermia following intraventricular injection of phenoxybenzamine in the pigeon at room temperature. Likewise, intraventricular phentolamine was shown to elevate *tb* in adult fowls at *ta* 6°C (Marley & Nisticò, 1975). This suggests that a greater release of NA from nerve terminals in the thermoregulatory pathway is needed in the maintenance of normal *tb* at *ta* around 20°C.

Variations in the location of the cannula might partly account for the variation of responses between different birds. However, no conclusions can be drawn without more accurate mapping. The locations of cannulae in pigeons that died were rather deep, bordering on optic chiasm and optic tract. The cause of death remains unestablished.

In conclusion, these experiments demonstrate that hypothalamic injection of phentolamine can cause a considerable attenuation of the hypothermic effects of an intramuscular injection of NA in the pigeon at *ta* 6°C. The results further suggest that the hypothermic effect of peripheral NA is mainly of central origin and due to an α-adrenoceptor agonist effect in the hypothalamus.

Please send reprint requests to A.P.

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