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ADRENOMEDULLARY FUNCTION IN THE NEONATAL RAT: RESPONSES TO ACUTE HYPOXIA

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SUMMARY

1. The mechanism of release of catecholamines from the adrenal medulla of neonatal rats was examined, together with the role of these amines in the ability of the organism to withstand acute O_2 deprivation.

2. Splanchnic innervation of the rat adrenal is non-functional until the end of the first postnatal week. Nevertheless, hypoxia caused depletion of adrenal catecholamines in 1-day-old rats as well as in 8-day-old animals. Pre-treatment with cholinergic receptor blocking agents did not prevent the catecholamine response at 1 day but did in older animals; these results indicate that the depletion mechanism is not neurogenic in 1-day-old animals but is neurogenic in 8-day-old animals. The proportions of noradrenaline and adrenaline released by hypoxic stress also differed at the two ages, with preferential release of adrenaline by the neurogenic mechanism but not by the non-neurogenic one.

3. The ontogenetic replacement of non-neurogenic adrenomedullary responses by the neurogenic mechanism was directly related to the onset of splanchnic nerve function. Treatments which accelerated the development of neuronal connexions (neonatal hyperthyroidism, maternal stress) caused premature loss of the non-neurogenic response.

4. Prior to the development of sympathetic nerve function, adrenal catecholamines play a predominant role in enabling the neonate to survive hypoxia. Interference with the release of adrenal amines invariably increased mortality during hypoxia. In contrast, interference with sympathetic neural release of catecholamines did not affect the ability of 1-day-old rats to withstand hypoxia, indicating that survival during low P_{O_2} conditions is not dependent on the sympathetic innervation at that stage of development. After functional development of the sympathetic nerves and disappearance of non-neurogenic adrenomedullary responses, the neonatal rats became partially dependent upon catecholamines derived from sympathetic terminals; administration of bretylium at 8 days significantly compromised survival during hypoxia.

5. Interference with adrenergic receptor function also interfered with the ability of neonatal rats to withstand low P_{O_2}. At 1 day of age, either phenoxybenzamine or ICI-118551, but not atenolol, shortened the survival time during hypoxia. At 8 days, only phenoxybenzamine did so.

6. These results indicate that the ability of the neonatal rat to survive acute hypoxia depends upon the release of catecholamines and their subsequent actions at peripheral α- and β_2-adrenergic receptors; in the absence of a functional sympathetic innervation, the amines are released from the adrenal medullae by some other mechanism, which disappears shortly after birth.

INTRODUCTION

The sympathetic nervous system and its endocrine counterpart, the adrenal medulla, are known to play an important role in eliciting physiological responses to stress situations. In the fetus and neonate, adrenergic mechanisms are called into play to produce the metabolic, cardiovascular and respiratory adjustments which are required during the perinatal period (Jones, 1980; Silver & Edwards, 1980). In the mature animal, sympathetic neurones are of major significance in physiological control; in contrast, in the fetus and neonate, activity of the adrenal medulla may be of relatively greater importance because the development of the sympathetic nervous system is incomplete (Pappano, 1977; Gootman, Buckley & Gootman, 1979). Studies with new-born humans indicate that a surge of sympatho-adrenal activity is associated with vaginal delivery and interference with the catecholamine surge is associated with decreased survival potential (Lagercrantz & Bistoletti, 1973; Nylund, Lagercrantz & Lunell, 1979). Be that as it may, it is difficult to distinguish between contributions provided by sympathetic neurones and those from the adrenal medullae, since activation of both occurs during stress. For this reason, the rat provides a convenient model in which to study the role of the adrenal medulla in neonatal physiology. In this species, sympathetic innervation of autonomic end-organs is absent or non-functional at birth (Slotkin, Smith, Lau & Bareis, 1980; Slotkin, 1984*a*, *b*) and the release of adrenomedullary catecholamines provides the major route for adrenergic effects. The splanchnic nerve, which ordinarily controls adrenomedullary secretion, is also not functional until the end of the first postnatal week (Slotkin, 1973*a*; Bartolome & Slotkin, 1976; Bareis & Slotkin, 1978) but catecholamines do appear to be secreted non-neurogenically in response to at least some pharmacological stimuli (Anderson & Slotkin, 1975; Bartolome, Bartolome, Seidler, Anderson & Slotkin, 1976; Bartolome & Slotkin, 1976; Chantry, Seidler & Slotkin, 1982).

In order to demonstrate whether non-neurogenic adrenal catecholamine release is of physiological significance to the neonate, it is necessary to show that a secretory response occurs during a non-pharmacological environmental challenge. In the calf, hypoxia or asphyxia have been shown to be potent stimuli for adrenomedullary secretory responses in the perinatal period; these effects also appear to be mediated non-neurogenically, as cutting the splanchnic nerve fails to prevent the responses (Comline & Silver, 1966; Silver & Edwards, 1980). A second requirement is to demonstrate that the catecholamines derived from the adrenal medulla are essential to the neonate's ability to tolerate the stress. In the current study, we have examined: (a) the mechanisms of catecholamine secretion which operate in the neonatal rat in response to acute hypoxia, (b) the relative roles played by adrenal and neuronal catecholamines and (c) the requirement for adrenal catecholamine activation of

various adrenergic receptor populations. Finally, we have addressed the major question of the mechanism by which the specialized secretory process disappears as the nervous system matures.

METHODS

Experimental procedures. Pregnant Sprague–Dawley rats (Zivic–Miller, Allison Park, PA, U.S.A.) were housed individually in plastic breeding cages and allowed free access to water and food. Neonates were randomized at birth, redistributed to nursing dams to minimize litter-to-litter variations and maintained at a litter size of ten to eleven pups to ensure a standard nutritional status; randomization was repeated at 2–3 day intervals and for each experiment, rats were chosen from several different cages. Animals were placed in a 22 l closed container kept at 37 °C in an incubation bath and hypoxia was produced by ventilating the container with pre-warmed (37 °C), humidified gas mixtures (5% or 7% O_2 balanced with N_2) at a rate of 15 l/min; under these conditions the P_{O_2} of source and exhaust gases was the same. Barometric pressure during the experiments ranged from 759 to 763 mmHg. Control animals were placed in an identical container ventilated with warmed, humidified room air. At the end of the hypoxic challenge, the chamber was flushed with room air, animals were killed by decapitation and the adrenals prepared for catecholamine determinations (see below) or, in studies with more prolonged exposure, the animals were counted to establish the rate of mortality; in the latter case the pups were allowed a 30 min recovery period in the incubator before the number of survivors was tabulated.

For studies utilizing pharmacological agents, drugs were dissolved in water and, unless noted otherwise, administered subcutaneously 15 min prior to low P_{O_2} exposure; controls received equal volumes of water (1 μl/g). Surgical procedures (bilateral adrenalectomy or sham operation) were performed under ether anaesthesia. A small incision was made dorsal to the adrenal gland which was then exposed and extirpated. Sham operations were carried out identically except that the glands were not removed.

Development of cardiac sympathetic function was assessed by the ability of the myocardium to respond biochemically to reflex sympathetic stimulation evoked by insulin. Previous studies have shown that acute elevation of cardiac ornithine decarboxylase activity caused by hypoglycaemic stress requires effective sympathetic neurotransmission from the central nervous system to the heart and thus provides a reliable index of development of cardiac–sympathetic synaptic function (Bartolome, Lau & Slotkin, 1977; Lau & Slotkin, 1979*a*; Slotkin & Bartolome, 1983); the advantage of this biochemical assessment is that the evaluation can be done in intact, unanaesthetized, unoperated animals which are left with their mothers and control litter-mates throughout the procedure. Hypoglycaemic stress was initiated by administering 20 i.u./kg of insulin s.c. and animals were killed 3 h later. Hearts were then excised and used for ornithine decarboxylase determinations.

Assay procedures. Adrenals were homogenized in 0·1 N-perchloric acid using a close-fitting ground-glass homogenizer and the samples were centrifuged at 26000 *g* for 10 min to remove precipitated protein. The supernatant solution was used for analysis of total adrenal catecholamines by the trihydroxyindole method of Merrills (1963) using an autoanalyzer and adrenaline as a standard. When separate analyses of noradrenaline and adrenaline were required, the amines were separated by reverse-phase high performance liquid chromatography (h.p.l.c.) and analysed by electrochemical detection as described previously (Seidler & Slotkin, 1981). Values were accurate to 0·01 ng by the h.p.l.c. procedure and to 1 ng by the fluorescence measurement; duplicate samples differed by $<1\%$.

Serum corticosterone was measured by radioimmunoassay using antiserum supplied by Radioassay Systems Laboratories, Inc. (Carson, CA, U.S.A.). Samples were extracted with ethyl acetate (1 ml/10–100 μl of serum), centrifuged, evaporated under N_2 and corticosterone assayed in the dried extract. Values obtained were accurate to within 0·1 ng/ml and duplicate samples differed by $<1\%$.

For determination of ornithine decarboxylase activity, hearts were homogenized in nineteen volumes of 10 mM-Tris (pH 7·2), centrifuged at 26000 *g* for 20 min and the supernatant assayed as described by Lau & Slotkin (1979*b*). This method was accurate to 0·01 nmol/g.h and duplicates were reproducible to within 3%.

Statistics. Data are reported as means and standard errors (catecholamine, corticosterone and ornithine decarboxylase determinations) with significance calculated using the Student's t test (two-tailed, unpaired); values were obtained only from animals surviving hypoxic exposure. Non-parametric variables (mortality data) were compared using Fisher's exact test. In both cases, differences were considered significant at a level of $P \leqslant 0{\cdot}05$.

Materials. Chlorisondamine chloride and desoxycorticosterone pivalate were obtained from CIBA Pharmaceuticals (Summit, NJ, U.S.A.), 6-hydroxydopamine hydrobromide, 2-deoxyglucose, 3,3',5-triiodo-L-thyronine sodium salt and atropine sulphate from Sigma Chemical Co. (St. Louis, MO, U.S.A.), bretylium tosylate from Amar-Stone Del Caribe (Puerto Rico), atenolol hydrochloride from Stuart Pharmaceuticals (Wilmington, DE, U.S.A.), ICI-118551 from Imperial Chemical Industries PLC (Cheshire), phenoxybenzamine hydrochloride from Smith, Kline and French (Philadelphia, PA, U.S.A.), insulin from Eli Lilly Corp. (Indianapolis, IN, U.S.A.) and [1-^{14}C]L-ornithine (specific activity 53 mCi/mmol) from New England Nuclear Corp. (Boston, MA, U.S.A.). All dosages refer to the salt form indicated.

RESULTS

Adrenal catecholamine depletion caused by neonatal hypoxia (Table 1)

Acute exposure to low P_{O_2} caused significant depletion of adrenal catecholamines in neonatal rats. In 1-day-old animals, in which the splanchnic innervation had not yet developed, exposure to either 7 % O_2 for 120 min or 5 % O_2 for 75 min produced a net loss of at least 20 % of the total catecholamine content. To assess whether there was preferential release of either noradrenaline or adrenaline, the ratio of the two amines was compared after exposure to either room air or 7 % O_2; no difference was seen. The non-neurogenic nature of the response in 1-day-old animals was confirmed by studies utilizing chlorisondamine; pre-treatment with this nicotinic receptor antagonist totally blocks adrenomedullary secretion in mature animals and, in the dose utilized here, is fully effective in blocking nicotinic receptors in the neonatal adrenal (Bartolome *et al.* 1976). 24 h after birth, however, chlorisondamine failed to prevent hypoxia-induced catecholamine loss. Likewise, pre-treatment with atropine, a muscarinic receptor antagonist, failed to block this response.

These results were contrasted with those obtained in 8-day-old animals. Again, hypoxia evoked significant catecholamine loss from the adrenals, but in this case there was preferential release of adrenaline as evidenced by a rise in the ratio of noradrenaline/adrenaline remaining in the tissue; it is unlikely that this change represents instead a specific, rapid replenishment of noradrenaline, as replacement of amine storage vesicles lost during neurogenic release (which requires 24 h) must first occur (Winkler, 1977). The adrenomedullary response at 8 days was clearly neurogenic, since pre-treatment with chlorisondamine completely prevented the loss of catecholamines caused by 7 % O_2; blockade of the neurogenic response in the 5 % O_2 group at 8 days of age resulted in 100 % mortality within 25 min.

Hypoxia-induced mortality: interference with catecholamine release

The finding that prevention of the adrenomedullary catecholamine response to hypoxia resulted in death of 8-day-old rats suggested that the gland plays an important role in survival during low O_2 conditions. Although 60 min of hypoxia did not result in any deaths in 1-day-old rats, extending the period of exposure to 5 % O_2 beyond 75 min resulted in a substantial incidence in mortality (Fig. 1); few of these

TABLE 1. Effects of hypoxia on adrenal catecholamines in 1 and 8-day-old rats

Age and exposure	Pre-treatment: Control (catecholamine levels, ng/gland)	Chlorisondamine (catecholamine levels, ng/gland)	Atropine (catecholamine levels, ng/gland)
1 day old			
Room air	294±8	294±6	322±14
Noradrenaline/adrenaline	0·29±0·01		
7% O_2, 120 min	235±4*	239±8*	—
Percentage depletion	20±1	19±3	
Noradrenaline/adrenaline	0·30±0·01		
5% O_2, 75 min	236±12*	218±14*	263±10*
Percentage depletion	20±4	26±5	20±3
8 days old			
Room air	1020±40	1000±20	
Noradrenaline/adrenaline	0·25±0·02		
7% O_2, 120 min	632±44*	960±48	
Percentage depletion	38±4	4±5	
Noradrenaline/adrenaline	0·32±0·02*		
5% O_2, 50 min	730±80*	—	
Percentage depletion	28±8		

Data represent mean±S.E. of mean of values from seven to twenty rats in each group. Asterisks denote significant differences from comparably pre-treated room air group. Chlorisondamine (5 mg/kg, S.C.) or atropine (2·5 mg/kg, S.C.) were administered 15 min before hypoxic exposure. Controls received an equal volume of water (1 μl/g). 8-day-old animals in the 5% O_2, chlorisondamine pre-treated group all died within 25 min of commencing hypoxic conditions.

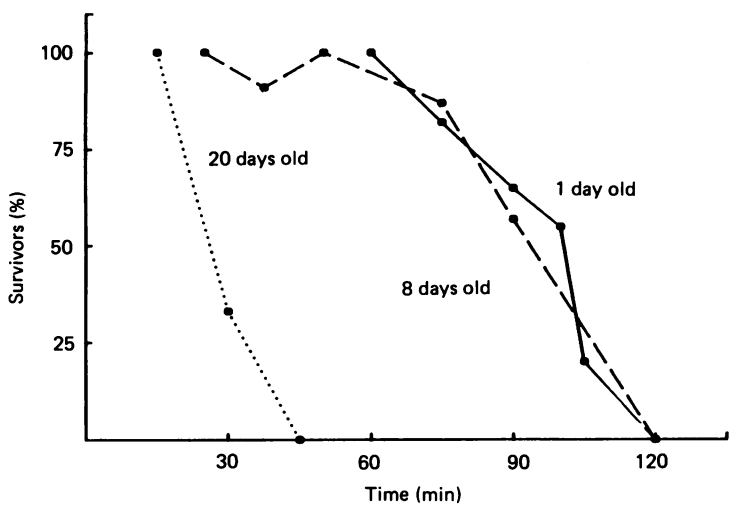

Fig. 1. Effects of exposure to 5% O_2 on survival in 1, 8 and 20-day-old rats. Points represent percentage of animals surviving, determined from eleven to ninety-nine animals at each time point for 1-day-old rats, ten to fifty-seven for 8-day-old rats and six for 20-day-old rats.

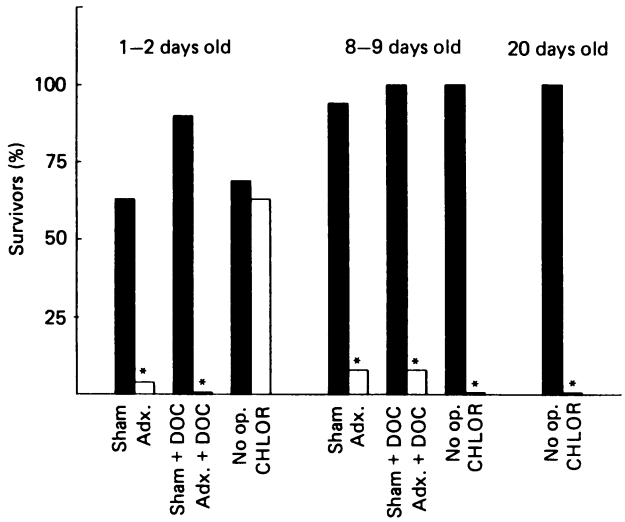

Fig. 2. Effects of adrenalectomy, corticosterone and chlorisondamine pre-treatment on survival during hypoxia. Sham, sham operation; adx., bilateral adrenalectomy; DOC, desoxycorticosterone (17 mg/kg I.P.); no op., no operation; CHLOR, chlorisondamine (5 mg/kg S.C.). Bars represent percentage of animals surviving hypoxia, determined from thirteen to sixteen animals in each group for 1-day-old rats, seven to twenty-eight for 2-day-old rats, fourteen for 8-day-old rats, ten to twenty-six for 9-day-old rats and six to ten for 20-day-old rats. Asterisks denote significant differences from appropriate control. Chlorisondamine studies were performed at 1, 8 and 20 days of age. In studies with surgical procedures, operations were performed on days 1 and 8 and the animals tested 24 h later; neither surgery nor drug pre-treatments (alone or combined) produced mortality in animals exposed to room air during the test period. Hypoxic conditions were: 1–2-day-old rats, 5 % O_2 for 90 min; 8–9-day-old rats, 5 % O_2 for 37·5 min; 20-day-old rats, 5 % O_2 for 15 min.

animals survived exposure for longer than 100 min. 8-day-old rats exhibited a similar degree of mortality during hypoxia but older animals (20 days) were much more sensitive to 5 % O_2, with no survivors past 45 min.

Since chlorisondamine could not be used to prevent catecholamine release and hence establish the role of catecholamines at 24 h post partum, we examined the effects of bilateral adrenalectomy. When the adrenals were removed at 1 day of age and animals tested 24 h later, there was a substantial increase in hypoxia-induced mortality (Fig. 2); two-thirds of sham-operated animals survived exposure to 5 % O_2 for 90 min, whereas mortality was almost total in the adrenalectomized group. To ensure that it was the loss of adrenomedullary, and not adrenocortical function that was responsible for the deleterious effect on survival, sham-operated and adrenalectomized rats were given a massive (17 mg/kg I.P.) replacement dose of desoxycorticosterone 15 min prior to hypoxic challenge; administration of this steroid failed to protect adrenalectomized rats from hypoxia. (The predominant steroid in the rat adrenal is corticosterone, which is primarily a mineralocorticoid; consequently, desoxycorticosterone, a similar but more potent and longer-acting steroid, was chosen for replacement.) The drastic effects of adrenalectomy in 1-day-old rats can be

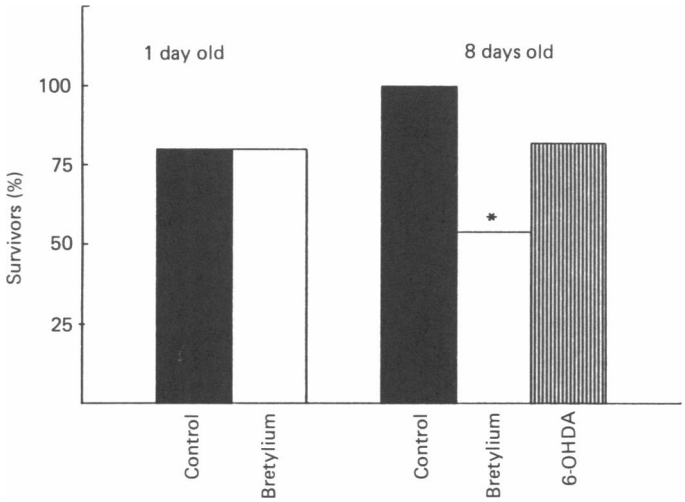

Fig. 3. Effects of bretylium and 6-hydroxydopamine on survival during hypoxia. Bars represent percentage of animals surviving hypoxia, determined from ten animals in each group for 1-day-old rats and ten to twenty-two for 8-day-old rats. Asterisk denotes significant difference from comparable age control. Neither bretylium pre-treatment (10 mg/kg s.c. given just before hypoxic exposure) nor 6-hydroxydopamine (6-OHDA) (100 mg/kg s.c. on postnatal days 1, 2 and 3) caused mortality in animals exposed to room air. Hypoxic conditions were: 1-day-old rats, 5% O_2 for 90 min; 8-day-old rats, 5% O_2 for 37·5 min.

contrasted with the absence of any influence on mortality rate in unoperated animals given chlorisondamine. Similar studies were conducted in rats operated upon at 8 days of age and challenged at 9 days, in this case using an exposure time of 37·5 min at 5% O_2 (Fig. 2). Nearly all the sham-operated animals survived the low P_{O_2} conditions but few if any in the adrenalectomized group were alive even after this short period. Again, massive doses of desoxycorticosterone did not protect adrenalectomized rats from hypoxia. Prevention of adrenomedullary catecholamine release with chlorisondamine pre-treatment produced an increase in mortality in the older rats (8 day or 20-day-old animals) which was equivalent to that seen with bilateral adrenalectomy.

In the mature animal, adrenomedullary and sympathetic neuronal catecholamines are often released by the same stimuli. To determine whether adrenomedullary function predominates in the neonate, we examined the influence of two agents which specifically interfere with the neuronal component of adrenergic effect: bretylium, which prevents neuronal release of noradrenaline but not adrenomedullary release of catecholamines, and 6-hydroxydopamine, which destroys sympathetic nerve terminals without affecting the chromaffin cells of the adrenal medulla (Kostrzewa & Jacobowitz, 1974). Pre-treatment of 1-day-old rats with bretylium (10 mg/kg s.c., given 15 min prior to a 90 min exposure to 5% O_2) did not influence the incidence of survival (Fig. 3). In contrast, 8-day-old rats given the same pre-treatment and exposed to low O_2 (5% for 37·5 min) showed a substantial and significant increase in mortality. The effects of early postnatal destruction of sympathetic nerve terminals by 6-hydroxydopamine (100 μg/kg s.c., given daily on days 1, 2 and 3) were

also evaluated; this regimen has been shown to cause loss of over 90 % of sympathetic nerve terminals in the neonate (Lau, Burke & Slotkin, 1982). 6-Hydroxydopamine produced a small increase in hypoxia-induced mortality in 8-day-old animals, but the effect was not as great as that seen with acutely administered bretylium.

Hypoxia-induced mortality: interference with catecholamine effects

Selective antagonists were used to evaluate the receptor subtypes participating in the adrenergic responses (Fig. 4). Pre-treatment with ICI-118551 (10 mg/kg s.c.,

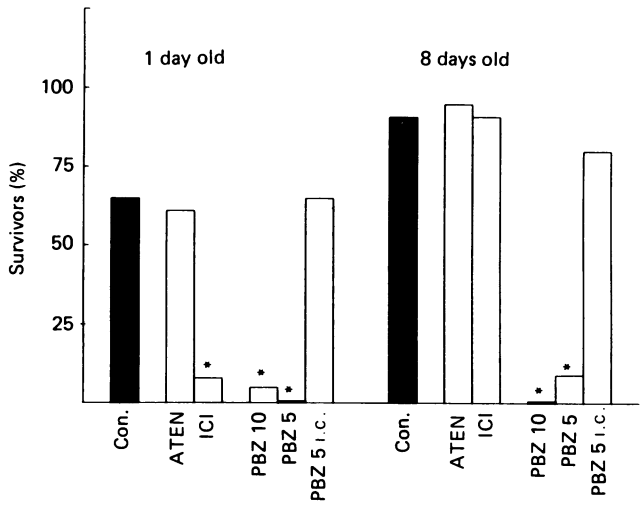

Fig. 4. Effects of adrenergic receptor blockade on survival during hypoxia. Con., control; ATEN, atenolol (10 mg/kg s.c.); ICI, ICI-118551 (10 mg/kg s.c.); PBZ 10, phenoxybenzamine (10 mg/kg s.c.); PBZ 5, phenoxybenzamine (5 mg/kg s.c.) and PBZ 5 I.C., phenoxybenzamine (5 μg/g brain weight given intracisternally, I.C.). Bars represent percentage of animals surviving hypoxia, determined from eleven to forty-eight animals in each group for 1-day-old rats and ten to forty for 8-day-old rats. Asterisks denote significant differences from control; control groups gave identical mortality rates whether vehicle was injected s.c. or I.C., so the value present is an average for both vehicle treatment routes. Drug pre-treatments did not cause mortality in animals exposed to room air. Hypoxic conditions were: 1-day-old rats, 5 % O_2 for 90 min; 8-day-old rats, 5 % O_2 for 37·5 min.

given 15 min before a 90 min exposure to 5 % O_2), a β-receptor antagonist selective for the β_2-receptor subtype, significantly reduced the ability of neonates to withstand low P_{O_2} conditions. In contrast, atenolol (10 mg/kg s.c.), a cardio-selective β_1-receptor antagonist, had no apparent effect. Phenoxybenzamine (5 or 10 mg/kg s.c.), an irreversible α-receptor blocking drug, had a drastic effect, reducing the survival rate almost to none. Receptor blocking agents given to 8-day-old rats exhibited a slightly different spectrum of action (Fig. 4): as in 1-day-old rats, systemic administration of phenoxybenzamine reduced survival during hypoxia (37·5 min at 5 % O_2) and atenolol was ineffective; however, unlike the younger rats, the adverse effects of ICI-118551 were not apparent.

The marked increase in hypoxia-induced neonatal mortality after phenoxybenzamine treatment raised the possibility that many of the vital physiological mechanisms brought into play by hypoxia-induced secretion of adrenal catecholamines involve α-adrenergic receptors. First, adrenal catecholamines could promote survival during hypoxia by a central mechanism which could then be prevented by phenoxybenzamine; since the blood–brain barrier is immature in the neonatal rat, both catecholamines and phenoxybenzamine may enter the central nervous system (Kostrzewa & Jacobowitz, 1974). However, direct introduction of equivalent doses of phenoxybenzamine directly into the cerebrospinal fluid (5 μg/g brain weight, given intracisternally) failed to cause any increase in mortality at either 1 or 8 days of age (Fig. 4); few of the litter-mates given phenoxybenzamine systemically at the same dosage level (5 mg/kg body weight) survived hypoxic challenge.

Because α-receptors also participate in the release of adrenocorticotrophin (ACTH) from the pituitary (Giguere, Cote & Labrie, 1981), phenoxybenzamine could conceivably exert an indirect effect on the ability to survive hypoxia by impairing the secretion of adrenal steroids. Direct measurement of serum corticosterone did indicate a surge in activity accompanying hypoxia in rats at either 1 day of age (room air = $15{\cdot}8 \pm 2{\cdot}1$ ng/ml, after 5% O_2 for 50 min = $52{\cdot}2 \pm 1{\cdot}2$ ng/ml, $P < 0{\cdot}001$; values are means ± S.E. of eight to ten rats in each group) or at 8 days (room air = $4{\cdot}7 \pm 0{\cdot}7$ ng/ml, after 5% O_2 for 15 min = $10{\cdot}8 \pm 1{\cdot}1$ ng/ml, $P < 0{\cdot}001$). However, phenoxybenzamine (10 mg/kg S.C. given 15 min before initiating hypoxia) actually raised circulating steroid levels whether or not the neonates were hypoxic (1 day old, phenoxybenzamine alone = $41{\cdot}6 \pm 1{\cdot}6$ ng/ml, phenoxybenzamine + hypoxia = $57{\cdot}9 \pm 1{\cdot}5$ ng/ml; 8 days old, phenoxybenzamine alone = $9{\cdot}8 \pm 1{\cdot}6$ ng/ml, phenoxybenzamine + hypoxia = $12{\cdot}1 \pm 0{\cdot}1$ ng/ml; all values $P < 0{\cdot}002$ *vs.* room air controls of comparable age). It is therefore unlikely that indirect effects on adrenal corticosteroids are involved in the adverse actions of this drug.

Peripheral effects mediated through α- and/or β_2-receptors are therefore likely to predominate. Both α- and β_2-adrenergic receptors are intimately involved in altering respiratory, cardiovascular and metabolic status in response to surges in circulating catecholamines during neonatal stress (Jones, 1980). If these alterations are mainly respiratory, then during exposure to total anoxia, adrenergic antagonists should produce no additional mortality. As shown in Fig. 5, total anoxia severely shortened survival time, and ICI-118551 had no mortality-promoting effect under these conditions. In contrast, the deleterious actions of phenoxybenzamine were still partially apparent. Similarly, 2-deoxyglucose was used to determine whether its interference with glucose utilization produced effects which were additive to those seen with adrenergic blocking agents. Administration of 2-deoxyglucose (2 g/kg I.P., 15 min pre-treatment) compromised the ability of 1-day-old rats to withstand hypoxia (Fig. 6) but again, phenoxybenzamine still caused an additional impairment.

Hypoxia-induced mortality: acceleration of onset of neuronal function

The disappearance of non-neurogenic adrenomedullary catecholamine secretion occurs at the time at which sympathetic neurones become functional (Comline & Silver, 1966; Slotkin, 1973*a*; Bartolome & Slotkin, 1976; Bareis & Slotkin, 1978). If

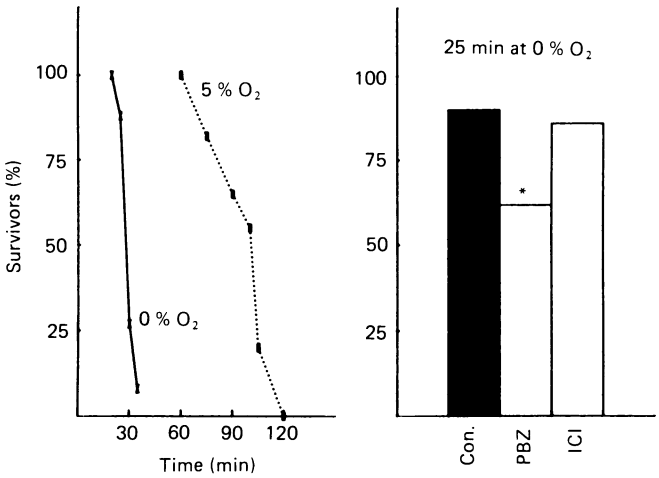

Fig. 5. Comparison of survival times in 1-day-old rats in 5 % or 0 % O_2 (left) and effects of adrenergic blockade during anoxia (right). Con., control; PBZ, phenoxybenzamine (10 mg/kg s.c.); ICI, ICI-118551 (10 mg/kg s.c.). Points and bars represent percentage of animals surviving hypoxia (left) or anoxia (left and right), determined from twelve to seventy-one rats at each time point for 0 % O_2, ten to ninety-nine for 5 % O_2 and thirty-four to fifty-two for the drug treatments. Asterisk denotes significant difference from anoxic control.

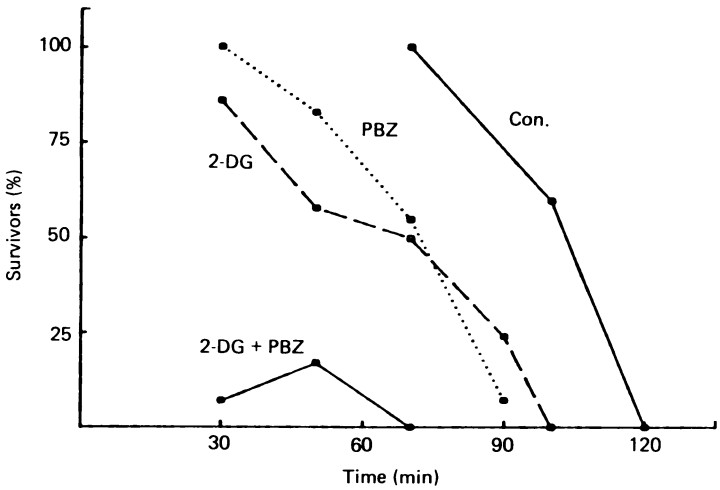

Fig. 6. Effects of 2-deoxyglucose and phenoxybenzamine on survival in 1-day-old rats exposed to 5 % O_2. Con., control; PBZ, phenoxybenzamine; 2-DG, 2-deoxyglucose. Points represent percentage of animals surviving hypoxia, determined from ten to twenty-four rats at each time point. Two control groups were utilized, one receiving 2 g/kg dextrose I.P. (control for 2-deoxyglucose group) and the other receiving saline S.C. (control for phenoxybenzamine group). Since control groups gave identical mortality rates, the control curve given represents pooled data of the two groups. Neither 2-deoxyglucose (2 g/kg I.P.) nor phenoxybenzamine (10 mg/kg S.C.) caused mortality in animals exposed to room air.

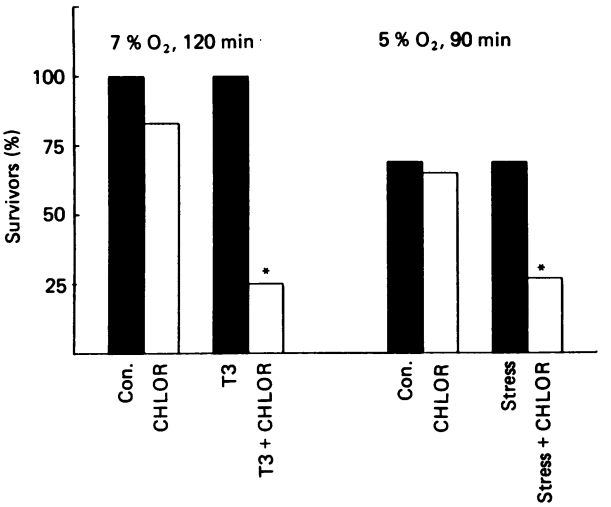

Fig. 7. Effects of neonatal hyperthyroidism and maternal stress on survival of 1 and 2-day-old rats exposed to hypoxia. Con., control; CHLOR, chlorisondamine (5 mg/kg s.c.); T3, triiodothyronine (0·1 mg/kg s.c.) and stress, maternal stress. Chlorisondamine was given 15 min, and T3 24 h, before commencing hypoxia. Maternal stress was produced by alkaline saline injections (1 ml/kg s.c.) for the last 5 days of pregnancy. Hypoxia was initiated at 2 days of age in the T3 study and 1 day of age in the stress study. Bars represent percentage of animals surviving hypoxia, determined from eleven to sixteen animals in each group for 1-day-old rats and twelve for 2-day-old rats. Asterisks denote significant differences from comparable control treatments. Neither drug nor stress (alone or combined) caused mortality in animals exposed to room air.

these events are linked, then acceleration of neuronal development should produce a premature loss of the non-neurogenic mechanism and a consequent appearance of chlorisondamine sensitivity during hypoxic challenge. 1-day-old rats were given triiodothyronine (0·1 mg/kg s.c.) or equivalent injections of vehicle and used for studies of hypoxia-induced mortality 24 h later. Although this hormone treatment does not accelerate chromaffin cell development, it does cause premature onset of functional sympathetic innervation within this time span (Lau & Slotkin, 1979*a*; Slotkin, 1984*a*, *b*). The ability of rats given triiodothyronine to withstand exposure to 7 % O_2 was not impaired (Fig. 7); however, chlorisondamine treatment just prior to exposure to low P_{O_2} resulted in virtually total mortality in the hyperthyroid group but not in controls subjected to the same degree of hypoxia.

A second means of accelerating sympathetic nerve development is to subject pregnant rats to stress during the last trimester (Smith & Mills, 1983). Maternal stress was produced by daily injections of alkaline saline (1 mg/kg s.c. at pH 9–10) for the last 5 days of pregnancy. We confirmed that the sympathetic innervation of the heart was indeed functional at 1–2 days of age in the offspring of stressed dams as assessed biochemically after insulin challenge: in 1-day-old offspring of non-stressed rats, cardiac ornithine decarboxylase activity did not increase after insulin (basal activity = 2·25 ± 0·16 nmol/g.h, after insulin = 2·46 ± 0·20 nmol/g.h; values are

means ± S.E. of eight to fifteen rats in each group) but a substantial response was present in the stressed group (basal = 2·62 ± 0·24 nmol/g.h; after insulin = 4·99 ± 0·49 nmol/g.h, $P < 0·001$). Rats in the maternal-stressed group were able to tolerate hypoxia at 1 day of age (Fig. 7); however, when pre-treated with chlorisondamine these animals exhibited the increases in mortality characteristic of dependence upon neurogenic responses.

DISCUSSION

Despite the absence of sympathetic innervation, the chromaffin cells in the new-born rat adrenal contain all the necessary biochemical machinery to synthesize, store and release catecholamines (Slotkin, 1973*a*, *b*) and the tissue is not totally unresponsive to stressful stimuli (Anderson & Slotkin, 1975; Bartolome & Slotkin, 1976; Bartolome *et al.* 1976; Chantry *et al.* 1982); a variety of pharmacological agents have been shown to evoke catecholamine release in the neonate by a mechanism that appears to be totally independent of neuronal input and is therefore 'non-neurogenic' (Bartolome & Slotkin, 1976; Bartolome *et al.* 1976; Chantry *et al.* 1982). In the current study, exposure to low P_{O_2} was also found to elicit the same type of release. The differences between adrenomedullary secretion in the period before development of innervation and that in the post-innervation period can be best illustrated by the effects of nicotinic blockade, which prevents release in 8-day-old but not in 1-day-old rats. An additional difference in the non-neurogenic mechanism is its lack of specificity for either type of catecholamine: in 1-day-old rats noradrenaline and adrenaline were released in precisely the same proportion as that present in the adrenal, whereas in older animals there was preferential release of adrenaline in response to hypoxia. The latter finding is in keeping with the view that, when the sympatho-adrenal system matures, splanchnic nerve pathways can be selectively activated to produce release of either noradrenaline or adrenaline (Lewis, 1975); apparently, no such selectivity occurs with the non-neurogenic mechanism seen in the neonate.

The non-neurogenic secretory mechanism has been found to decline concurrently with the development of nerve competency (Comline & Silver, 1966; Slotkin, 1973*a*; Bartolome & Slotkin, 1976), raising the possiblity that it is neural activity *per se* which causes the loss of the unique ability of the immature adrenal to respond directly to stress. In the studies presented here, this hypothesis was tested by two paradigms in which sympathetic innervation was artificially accelerated. Administration of thyroid hormones is known to produce a widespread premature onset of function of sympathetic synapses, including those between the splanchnic nerve and chromaffin cells (Lau & Slotkin, 1979*a*, 1980; Chantry *et al.* 1982); functional neurotransmission is achieved within 24 h of a single injection of triiodothyronine to a 1-day-old rat (Lau & Slotkin, 1979*a*, 1980). When hyperthyroid neonates were exposed to 7% O_2 for 120 min, the neonates became sensitized to the effects of chlorisondamine, in that pre-treatment with the nicotinic blocking agent produced a substantial increase in mortality during the hypoxic challenge; this difference could not be attributed to direct effects of triiodothyronine on O_2 utilization, since mortality was unaffected in hypoxic neonates given the hormonal treatment without chlorisondamine. Thus, hyperthyroidism, which accelerates the onset of neuronal function, also eliminated

the capability of the neonates to withstand hypoxia by non-neurogenic mechanisms; instead, the animals were dependent upon synaptic activity and hence died when neurotransmission was blocked by chlorisondamine.

A second situation in which sympathetic activity may become elevated prematurely is after maternal stress during late pregnancy (Smith & Mills, 1983). In the present study, this was demonstrated by early onset of the ability of sympathetic nerves to stimulate cardiac ornithine decarboxylase activity; normally, this enzyme can be increased by sympathetic reflexes only after 1 week postnatally, since the response must await the development of functional sympathetic synapses (Bartolome *et al.* 1977; Bareis & Slotkin, 1980; Lau & Slotkin, 1980; Slotkin *et al.* 1980). After maternal stress however, neurally-derived response capabilities were apparent immediately post partum. Again, in 1-day-old rats in which nerve development was accelerated, there was a demonstrable dependency upon neurogenic input for survival during hypoxia, as chlorisondamine pre-treatment resulted in substantial mortality. Thus it appears that events which promote sympathetic neuronal function truncate the period in which non-neurogenic mechanisms are detectable.

The finding that, in older animals, blockade of adrenergic effect by chlorisondamine results in death during hypoxic challenge indicates that catecholamines provide essential activity required by such stress. A number of experiments conducted here indicate that the adrenal, in fact, plays a much more important role than does sympathetic innervation in the response to hypoxia in the neonate. In 1–2-day-old rats, eliminating adrenal catecholamines (bilateral adrenalectomy) prior to hypoxia resulted in loss of the ability to survive the stress, whereas interference with neuronal catecholamine release (bretylium pre-treatment) did not. In 8-day-old rats, in whom sympathetic innervation of autonomic effector tissues is functional (Bartolome *et al.* 1977; Bareis & Slotkin, 1978; Slotkin *et al.* 1980; Slotkin, 1984*a*, *b*), sensitivity to bretylium was pronounced; thus at 8 days the animals were dependent, in part, on neuronally-derived catecholamines. Studies conducted in 8-day-old animals after early (days 1–3) neonatal sympathectomy produced by 6-hydroxydopamine were less conclusive, probably due to the supersensitivity associated with chronic absence of sympathetic action and a corresponding increase in the effectiveness of catecholamines derived from the adrenal (Kostrzewa & Jacobowitz, 1974); more importance should be attached to the studies with acute bretylium treatment, where supersensitivity does not occur.

Because catecholamines are involved in a variety of respiratory, cardiovascular and metabolic adjustments to stress, it is difficult to assign a single specific role to the adrenomedullary amines released during neonatal hypoxia. However, a number of experiments conducted with adrenergic blocking agents gave at least some indications of selective effects. First, no increase in mortality occurred with atenolol (a β_1-blocker) pre-treatment prior to exposure to low P_{O_2} conditions. Since heart rate and contractility are regulated by β_1-receptors, it is tempting to conclude that there is no cardiac involvement in the effects of catecholamines during neonatal hypoxia; however, the neonatal heart has α-receptors which also are believed to participate in cardiac metabolism, inotropic responses and conduction (Govier, 1968; Benfey, 1973; Rabinowitz, Chuck, Kligerman & Parmley, 1975; Noguchi, Whitsett & Dickman, 1981; Clark, Patten & Filsell, 1982). Thus, it cannot be ruled out that catecholamines

released from the neonatal adrenal medulla during hypoxia may still have some impact on cardiac function. In contrast to the lack of effect of a β_1-antagonist on survival, β_2-blockade severely compromised hypoxic tolerance, but only in the immediate period post partum, as the blocking agent did not affect survival in 8-day-old rats. Lung compliance, resorption of lung fluid and production of surfactant are critical in establishing neonatal respiratory competence and are known to be responsive to circulating catecholamines via β-receptor mechanisms (Lawson, Brown, Torday, Madansky & Taeusch, 1978; Walters & Olver, 1978; Jones, 1980). Accordingly, the β_2-antagonist did not influence survival during total anoxia, a situation where effects on respiratory performance would not matter. It is thus likely that non-neurogenic adrenal catecholamine release affects neonatal respiratory function through actions on the β_2-receptor subpopulation.

A dramatic effect was seen with pre-treatment with an α-blocker prior to exposure to low P_{O_2}; the adverse actions of phenoxybenzamine resulted from peripheral adrenergic antagonism, since systemic but not central administration of the drug caused an increase in hypoxia-induced mortality. It is important that the reduced survival potential caused by phenoxybenzamine was apparent even in totally anoxic conditions or after interference with glucose utilization (2-deoxyglucose administration). Thus, it is likely that catecholamines released from the adrenal medulla act at a variety of sites to promote the ability of the neonate to survive hypoxia.

In conclusion, the neonatal rat is incapable of responding to hypoxic challenge through the types of adrenergic mechanisms which operate in the mature animal. Instead, adrenergic effects are mediated through a specialized adrenomedullary mechanism which releases catecholamines in the absence of nerve input. This mechanism disappears concurrently with the development of splanchnic nerve connexions to the tissue and events which accelerate the time course of nerve development lead to a correspondingly premature loss of non-neurogenic secretory capabilities. Interference with adrenal catecholamine release or blockade of the peripheral systemic effects of the amines result in inability of the neonate to survive hypoxia; a combination of respiratory, cardiovascular and metabolic actions appear to participate in the catecholamine-mediated response to hypoxia, chiefly involving α- and β_2-receptor populations in 1-day-old rats and exclusively α-receptors in 8-day-old rats.

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