Age-Related Cardiovascular Effects of Catecholamines in Anesthetized Piglets

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With the technical assistance of Isaac D. Frasier

SUMMARY We studied cardiac and peripheral circulatory effects of graded doses of catecholamines (0.05-1.0 µg/kg) in piglets aged ≤1 day, 2-4 days, 1 week, 2 weeks, and 2.5-3 months, under anesthesia with pentobarbital. We evaluated cardiovascular function from simultaneous recordings of aortic pressure, ventricular pressure and its first derivative, heart rate, and phasic carotid and femoral blood flows. We calculated vascular resistance as the ratio of mean aortic pressure to mean flow. The age of onset of a given cardiovascular response was determined, and magnitudes of each type of response were compared among the age groups. Norepinephrine elevated the blood pressure at all doses in piglets of all ages, elicited reflex bradycardia only in older piglets, and increased carotid resistance. Epinephrine elevated the blood pressure at all doses in piglets less than 1 week old, but low doses lowered the blood pressure when piglets older than 1 week of age; resistance changes in the femoral and carotid circulations were variable except in the 2.5-3 month age group. Isoproterenol elevated carotid resistance at all doses in piglets of all ages and increased heart rate at low doses in piglets older than 2 days of age; however, blood pressure and femoral resistance decreases were age and dose dependent. There were age-related differences in the catecholamine dose required to elicit a given cardiac or peripheral circulatory effect and age-related differences in the direction and magnitude of such effects. These results provide evidence for differing rates of postnatal maturation of cardiovascular α- and β-adrenergic mechanisms in swine. Circ Res 45: 282-292, 1979

THE NEONATAL pig is capable of central autonomic regulation of some cardiovascular functions when central vasomotor sites are stimulated directly (Gootman et al., 1972; Marshall and Breazile, 1974) or reflexly (Buckley et al., 1976; Gootman et al., 1978; Reddy et al., 1974). However, in our studies in piglets, the fully integrated heart rate, arterial pressure, and regional flow response was not observed until the piglets were at least 2 weeks old (Buckley et al., 1976; Gootman et al., 1972; Reddy et al., 1974). Reflex bradycardia was rarely seen at the peak blood pressure rise during stimulation of sciatic nerve afferents or carotid sinus receptors (Buckley et al., 1976), although bradycardia did occur in response to direct stimulation of nucleus ambiguus (Gootman et al., 1972, 1978). A fall in blood pressure during either central or afferent stimulation in piglets was difficult to obtain or was small (Buckley et al., 1976; Gootman et al., 1972, 1978; Marshall and Breazile, 1974). It seemed possible that these incomplete response patterns might be due, in part, to differences in maturity of cardiac and vascular effector systems in animals of different postnatal age. A survey of the literature convinced us that it would be arbitrary to extrapolate to swine the observations made on either the mature-at-birth lamb (Alexander et al., 1972; Assali et al., 1974; Friedman, 1972; Rudolph and Heyman, 1974) or the immature-at-birth dog (Boatman and Brody, 1967; Boatman et al., 1965; Gauthier et al., 1975; Geis et al., 1975; Privitera et al., 1969). There had been no report of observations on simultaneously measured cardiac and peripheral flow responses at different postnatal ages in any mammalian species, although this would be one approach to determining whether cardiac and peripheral vascular functions mature at the same rate.

We therefore undertook to examine postnatal maturation of adrenergic mechanisms by studying general cardiovascular responses to graded doses of catecholamines in piglets from birth to 3 months of age. The degree of functional maturity of adrenergic effector systems was assessed from changes in cardiac function and regional blood flows recorded simultaneously in the whole animal. Carotid and femoral circulations were chosen for study, as in our previous work (Buckley et al., 1976; Reddy et al., 1974), because of their different responsiveness to autonomic stimuli in adult mammals. The catecholamines selected were the neurotransmitter, norepinephrine; the adrenal medullary hormone, epinephrine, and the pharmacological β-receptor...
agonist, isoproterenol. Data obtained in this longitudinal age series of piglets were examined for age-related differences in the occurrence, direction, and magnitude of the responses. Evidence is presented here that adrenergic mechanisms in the heart and peripheral circulation exhibit differing degrees of immaturity during the first 2 weeks of postnatal life.

Methods
Animal Preparations
A total of 92 piglets aged 7 hours to 3 months, many of which were littermates, were used to evaluate catecholamine effects. All animals were anesthetized with sodium pentobarbital, given intraperitoneally: 10–15 mg/kg in piglets younger than 2 weeks of age and 20–30 mg/kg in older ones. Supplementary small doses (3–6 mg) were given intravenously as needed throughout an experiment. The right external jugular vein was catheterized and the catheter was advanced to the level of the right atrium. A continuous infusion of 5% dextrose in water was supplied to maintain hydration (50 ml/4 hour in young animals, 100 ml/4 hour in older ones). The abdominal aorta was catheterized through a femoral artery. Electromagnetic flow transducers (NarcoBio-Systems) were placed around the other femoral artery and a common carotid artery. Thoracotomy was performed through a midline incision after artificial ventilation was established with room air with a Palmer pump. The left or right ventricle was catheterized by direct puncture with a short stiff polyethylene catheter. In some piglets, an electromagnetic flow transducer (Carolina Medical Electronics) was placed around the pulmonary artery to determine cardiac output. Since the ductus arteriosus is often patent in piglets younger than 2 weeks of age (Evans et al., 1963), it was ligated when necessary.

Blood loss during all these procedures was minimal. Experiments lasted 3–4 hours, during which time the ECG also was monitored and body temperature, read at intervals from a rectal thermometer, was maintained when necessary by a warming lamp. Samples of arterial blood (0.5 ml) were taken hourly for blood gas and pH determinations in a Radiometer electrode assembly. As in our previous studies (Buckley et al., 1976; Reddy et al., 1974), blood gas composition was controlled by varying the depth of ventilation to preclude depression of cardiac effects of catecholamines by hypercarbia and acidosis (Downing et al., 1971) or release of catecholamines by hypoxia (Jones and Robinson, 1975). Decamethonium bromide (Burroughs-Wellcome Co.) was administered in doses of 0.5–1.0 mg/kg to minimize muscle tremor artifacts on ECG and flowmeter recordings. In piglets, this compound has only transient effects on the cardiovascular system (Crane et al., 1974) and does not alter efferent vagal discharge (Marshall and Breazile, 1974).

Recordings and Calculations
Figure 1 shows pressure and flow tracings recorded simultaneously with lead II of the ECG on a pair of oscilloscopic recorders (Electronics for Medicine, model PR-6). Aortic and intraventricular pressures were registered by calibrated Statham P23Db transducers. Mean aortic pressure (AoP, mm Hg) was determined by planimetric integration of the calibrated area shown in the upper part of Figure 1. The intraventricular pressure tracing was differentiated electronically by a calibrated RC circuit. Maximum dP/dt during contraction (dP/dt max, mm Hg/msec) was chosen as an index of myocardial contractility that would be relatively independent of changes in afterload (Mahler et al., 1975; van den Bos et al., 1973). Left or right ventricular end-diastolic pressure (EDP, mm Hg) was measured at the peak of the R-wave of the ECG. Mean femoral and carotid flows (FF, CAF, ml/min), and mean pulmonary flow when recorded as the measurement of cardiac output (CO, ml/min), were determined by planimetric integration of the calibrated areas shown in the lower part of Figure 1. Zero flows were recorded during the compensatory pause after extrasystoles induced by touching
the heart at intervals in an experiment and during occlusion of the vessel distal to the transducer at the end of an experiment. Transducers were calibrated in vitro with swine blood and vessels. Femoral and carotid resistances (Fem R, Car R) were calculated at the ratio of AoP to the respective oral and carotid resistances (Fem R, Car R) were calculated at the ratio of AoP to the respective mean flow and expressed in peripheral resistance units (PRU).

Experimental Protocols and Controls

Controls on pentobarbital anesthesia were carried out to assess the possibility of depression of cardiovascular function by that agent (Assali et al., 1974; Hornicke, 1966): (1) Baroreceptor reflex activity in 30 of the piglets was tested midway in the course of an experiment by carotid sinus inhibition during occlusion of both common carotid arteries for 20 seconds, as in our previous studies (Buckley et al., 1976). (2) Eight piglets in a separate group were decerebrated under diethyl ether anesthesia, allowed to blow off the ether, and used to test the AoP and heart rate (HR) responses to 0.1, 0.5, and 1.0 \( \mu \)g/kg doses of catecholamines; then the usual anesthetic dose of sodium pentobarbital was administered and catecholamine testing was repeated 15 minutes later. (3) Thirteen piglets in another group were anesthetized with 0.25% halothane in a mixture of \( N_2O \) and \( O_2 \), as in our previous studies (Buckley et al., 1976) and tested with graded doses of catecholamines. Sodium pentobarbital was then administered in the usual dose, halothane was discontinued, and catecholamine testing was repeated 15 minutes later.

Catecholamine experiments were carried out after initial control conditions of cardiovascular function had been established. Single doses of noradrenaline bitartrate (NE; Winthrop Laboratories), epinephrine-HCl (E; Parke-Davis Co.), or isoproterenol-HCl (ISP; Winthrop Laboratories) were injected in a random sequence through the jugular catheter. The dose range expressed as the base was 0.05–1.5 \( \mu \)g/kg for NE and E and 0.05–0.5 \( \mu \)g/kg for ISP. The latency of responses was measured to distinguish between direct and reflex effects. An interval of at least 5 minutes was allowed to elapse between each injection to permit return to control levels of function. Whenever the volume of injectate was 1 ml or more, the effects of a single injection of the same volume of physiological saline solution were evaluated as a further control. The contribution of \( \alpha- \) and \( \beta- \)adrenergic receptor mechanisms to the observed changes in peripheral resistances was evaluated with the aid of a fixed dose of each of two adrenergic blocking agents: 0.25 mg phentolamine/kg (PT; Ciba Pharmaceutical Co.) and 0.1 mg propranolol/kg (PRP; Ayerst Laboratories). Percent blockade was calculated as the ratio of the difference between responses to agonist (0.5 \( \mu \)g of NE per kg or 0.1 \( \mu \)g of ISP per kg), before and 10 minutes after injection of the blocking agent, and responses to the control. Some piglets were used for more than one experimental protocol.

Controls for vagus nerve activity were carried out after it became obvious in the earliest experiments that the youngest piglets did not exhibit bradycardia at the peak of the blood pressure rise after injection of NE. (1) Before producing adrenergic blockade at the end of the experimental protocol, bilateral vagotomy was performed in 42 of the piglets to determine whether the HR had been under parasympathetic influence. (2) The cardiac end of the cut right vagus nerve then was stimulated by conventional methods (1 mA, 2–10 Hz, 1.24-msec pulse duration, 10–20 second trains of stimuli) in 20 of these animals, of differing age, to determine whether cardiac responses could be elicited.

Statistical Methods

Piglets were grouped according to age: \( \leq 1 \) day, 2–4 days, 6–10 days (1 week), 12–17 days (2 weeks), and 2.5–3 months. Each one was its own control for all observations on effects of experimental interventions. In each, the maximum change in a given cardiovascular function was calculated as the change from an immediately preceding control recording.

Mean values (X) of initial and terminal control data were determined, together with their standard errors, and compared to ascertain the effect of the passage of time alone. Mean values of maximum changes (Xa) in a given cardiovascular function during or after a given experimental intervention were compared with zero change to establish the statistical significance of the observed change; cross-compared with respect to dose of administered test compounds; and cross-compared among age groups to determine age dependency of observed changes. These statistical comparisons were made by the appropriate -test (Armitage, 1971) performed on a programmable calculator (Hewlett-Packard, model 9820A). The null hypothesis for a two-tailed distribution was rejected at \( P \) values \( \leq 0.05 \).

Linear regression analysis was carried out to evaluate the correlations suggested by scatter plots. The accuracy of a correlation coefficient (r) was determined as its standard error, and the statistical significance of each was established (Armitage, 1971). The relationship between a change in a given cardiovascular function and the dose of a catecholamine was analyzed in each age group of piglets; only a few linear relationships were found (see Results). Since we were interested in direct vasoactive effects of catecholamines, we also examined the possibility that passive changes in vessel caliber, hence resistance, were produced by changes in AoP. In this situation, the changing CO would result in a change in peripheral flows. Therefore, we
examined the correlation between change in CAF or FF and change in AoP, and between change in CAF or FF and change in CO.

Results

Control Observations

Control Cardiovascular Function

Table 1 contains the mean values (X) and their standard errors of the initial control conditions and levels of cardiovascular function. The CO (not shown) ranged between 136 ± 15 and 178 ± 18 ml/ min per kg in piglets through 2 weeks of age. After a transient decline in HR and AoP and a decrease in peripheral flows at the time of thoracotomy, these functions stabilized again. The time course of AoP, HR, and LV or RV dP/dt max, observed during subsequent control periods between each experimental intervention, revealed decreasing function in only six individual piglets of different ages. However, the blood pressure levels in even these few piglets were still not suggestive of significant physiological deterioration.

Pentobarbital Anesthesia

The cardiovascular effects of a 3–6 mg supplemental dose of sodium pentobarbital, administered iv during the control periods, were transient and were not statistically significant. For example, in day-old piglets, AoP briefly decreased by 6.6 ± 3.0 mm Hg. Blood pressure rose in response to carotid artery occlusion at the midpoint of the experiment in animals of all ages, as was previously observed in piglets under halothane anesthesia (Buckley et al, 1976), and the increase ranged between +6.3 ± 1.3 mm Hg (P = 0.003) in day-old piglets and +9.3 ± 1.9 mm Hg (P = 0.005) in 2-week olds. The pressure increase was greater the older the animal and was accompanied by an increase in RV dP/dt max (from +0.05 ± 0.02 to +0.17 ± 0.05 mm Hg/msec) only in piglets 1 week old or older.

In the 13 piglets tested with catecholamines while under halothane and then pentobarbital anesthesia, there was no difference in the effects on AoP or HR; for example, the AoP increase after 1.0 /ig of E per kg was +40.5 ± 9.9 mm Hg under halothane, and then +37.5 ± 15 mm Hg under pentobarbital anesthesia.

In the eight decerebrate piglets, administration of 10–15 mg of sodium pentobarbital per kg lowered the AoP (Xa = -7.0 ± 1.5 mm Hg, P < 0.002) but decreased the HR in three piglets and increased it in three others. Responses to single doses of catecholamines were similar before and after the pentobarbital was given; for example, 0.5 /ig of NE per kg increased the AoP by 20 ± 4.2 mm Hg and then by 23.7 ± 7.3 mm Hg, respectively.

Catecholamine Experiments

Relationships between Peripheral Flows, AoP, and CO

Regression analysis showed that there was poor correlation between changes in CAF or FF and changes in AoP (r ranged between 0.37 and 0.45) in all groups of piglets given catecholamines. There was also poor correlation between changes in CAF or FF and changes in CO (r ranged between 0.14 and 0.43) during administration of NE or E in animals of any age. When changes in CO were produced by stimulation of vagal efferents to the heart, an experimental intervention that would not have a direct effect on vascular smooth muscle, a

### Table 1 Initial Control Conditions in Piglets Anesthetized with Pentobarbital

<table>
<thead>
<tr>
<th>Condition</th>
<th>1 day (n=14)</th>
<th>2-4 days (n=21)</th>
<th>1 week (n=24)</th>
<th>2 weeks (n=17)</th>
<th>2.5-3 months (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (kg)</td>
<td>X ± XE</td>
<td>X ± XE</td>
<td>X ± XE</td>
<td>X ± XE</td>
<td>X ± XE</td>
</tr>
<tr>
<td>Body temperature (°C)</td>
<td>X ± XE</td>
<td>X ± XE</td>
<td>X ± XE</td>
<td>X ± XE</td>
<td>X ± XE</td>
</tr>
<tr>
<td>Arterial P02 (torr)</td>
<td>X ± XE</td>
<td>X ± XE</td>
<td>X ± XE</td>
<td>X ± XE</td>
<td>X ± XE</td>
</tr>
<tr>
<td>Arterial PCO2 (torr)</td>
<td>X ± XE</td>
<td>X ± XE</td>
<td>X ± XE</td>
<td>X ± XE</td>
<td>X ± XE</td>
</tr>
<tr>
<td>Arterial pH</td>
<td>X ± XE</td>
<td>X ± XE</td>
<td>X ± XE</td>
<td>X ± XE</td>
<td>X ± XE</td>
</tr>
<tr>
<td>HR (beats/min)</td>
<td>X ± XE</td>
<td>X ± XE</td>
<td>X ± XE</td>
<td>X ± XE</td>
<td>X ± XE</td>
</tr>
<tr>
<td>LVEDP (mm Hg)*</td>
<td>X ± XE</td>
<td>X ± XE</td>
<td>X ± XE</td>
<td>X ± XE</td>
<td>X ± XE</td>
</tr>
<tr>
<td>LV dP/dt max* (mm Hg/msec)</td>
<td>X ± XE</td>
<td>X ± XE</td>
<td>X ± XE</td>
<td>X ± XE</td>
<td>X ± XE</td>
</tr>
<tr>
<td>RV EDP (mm Hg)†</td>
<td>X ± XE</td>
<td>X ± XE</td>
<td>X ± XE</td>
<td>X ± XE</td>
<td>X ± XE</td>
</tr>
<tr>
<td>RV dP/dt max† (mm Hg/msec)</td>
<td>X ± XE</td>
<td>X ± XE</td>
<td>X ± XE</td>
<td>X ± XE</td>
<td>X ± XE</td>
</tr>
</tbody>
</table>

* n = 8 for ≤1 day, 14 for 2-4 days, 16 for 1 week, and 11 for 2 weeks, measured after piglets were placed on artificial respiration and thoracotomized.

† n = 6 for ≤1 day, 7 for 2-4 days, 8 for 1 week, and 6 for 2 weeks, measured after piglets were placed on artificial respiration and thoracotomized.
positive correlation was obtained between changes in peripher al flows and changes in CO (r ranged between +0.51 ± 0.12 and +0.69 ± 0.10, P < 0.001). Thus, the results concerning peripheral blood flow and resistance changes presented below can be attributed to catecholamine actions on the respective peripheral circulations rather than on the CO.

Age-Related Differences in Occurrence and Pattern of Cardiac and Peripheral Responses to Catecholamines

Figure 2 presents bar diagrams of the mean changes in HR, dP/dt max, and AoP following administration of the smallest dose (0.05 μg/kg) of catecholamines in each age group of piglets.

Heart rate effects (Fig. 2, upper panels) were not observed in any piglet ≤1 day old given 0.05 μg of NE or E per kg but occurred in 25% of the piglets of this age given ISP. An increase in rate was seen consistently in 1- and 2-week-old piglets given 0.05 μg of NE per kg, in 2.5- to 3-month-old animals given 0.05 μg of E per kg, and in all piglets given this dose of ISP even at 2-4 days of age. Reflex bradycardia at the peak of the AoP increase after NE injection was observed only in 2.5- to 3-month-old piglets. Nevertheless, bilateral vagotomy resulted in stable tachycardia, increased right ventricular contractility, and elevated AoP in piglets through 2 weeks of age (Table 2, upper section). Furthermore, stimulation of the cardiac end of the right vagus nerve resulted in a significant bradycardia (Table 2, lower section).

Changes in RV dP/dt max occurred in all piglets given 0.05 μg of ISP per kg, but changes in LV dP/dt max were not observed in any piglet ≤1 day old given 0.05 μg of NE per kg (Fig. 2, middle panels). At the next highest dose (0.1 μg of NE per kg), responses were obtained in a few more animals in the two youngest age groups. The lack of a response is of physiological significance because left ventricular EDP did not change during NE administration, the mean initial control LV dP/dt max was the same in all age groups of piglets (Table 1), and there were significant increases in AoP to even the lowest dose of NE tested (Fig. 2, bottom panels). Since changes in AoP would have little or no effect on RV dp/dt max, the results of ISP administration provided clear evidence that a positive inotropic effect could be elicited in even the youngest piglets.

The AoP was altered significantly in every piglet given 0.05 μg of NE or E per kg (Fig. 2, bottom panels). There was an increase in AoP in response to NE at all ages. The effects of this low dose of E were particularly interesting: pressor responses occurred in piglets 4 days of age or younger, and AoP decreased (depressor response) in piglets 2 weeks old or older, whereas a mixture of these two types of responses occurred in piglets 1 week old. Only in piglets 1 week of age or older did ISP produce a depressor effect at this dose.

Table 3 summarizes the age-related differences in doses of NE (upper section) and ISP (lower section) that produced a response in a given cardiovascular function. Responses observed were both age and dose dependent. The change in diastolic blood pressure (DBP) was used as a further index of vascular reactivity. The difference between carotid and femoral circulatory effects of NE and ISP is particularly noteworthy.

The CAF generally increased in piglets given catecholamines, but there were some specific differences. A dose of 0.1 μg of ISP (or E) per kg was sufficient to elicit the flow response at all ages. Although this same dose of NE was sufficient to elicit such responses in piglets 4 days of age or younger, 0.25 μg of NE per kg was required in 1-week-old and 0.5 μg of NE per kg in 2-week-old animals. The Car R (Table 3) was elevated (range, 0.5-1.6 PRU) by doses of NE ≥ 0.25 μg/kg in piglets 4 days of age or younger, but not by any dose in older animals, and was lowered (range, 0.6-0.8 PRU) in all piglets given at least 0.1 μg of ISP per kg. The mean decrease in Car R in older animals given 0.05 μg of ISP per kg ranged from −1.8 ± 0.8 PRU (average 26%) at 1 week to −0.7 ± 0.1 PRU (average 56%) at 2.5–3 months.

The FF generally increased in piglets given cat-
CATECHOLAMINE ACTIONS IN PIGLETS/Buckley et al.

### Table 2 Effects of Bilateral Vagotomy and Efferent Vagal Stimulation in Piglets Anesthetized with Pentobarbital

<table>
<thead>
<tr>
<th>Age of piglets</th>
<th>≤1 day (n = 5)</th>
<th>2-4 days (n = 9)</th>
<th>1 week (n = 13)</th>
<th>2 weeks (n = 12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bilateral vagotomy</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>∆ HR (beats/min)</td>
<td>+17 ± 5*</td>
<td>+15 ± 3*</td>
<td>+17 ± 3*</td>
<td>+31 ± 6*</td>
</tr>
<tr>
<td>∆ RV dP/dt max (mm Hg/msec)</td>
<td>+0.4 ± 0.1</td>
<td>+0.4 ± 0.1*</td>
<td>+0.4 ± 0.2*</td>
<td>+0.3 ± 0.1*</td>
</tr>
<tr>
<td>∆ AoP (mm Hg)</td>
<td>+6.6 ± 3.5</td>
<td>+16.5 ± 3.8*</td>
<td>+13.5 ± 3.2*</td>
<td>+14.3 ± 3.8*</td>
</tr>
<tr>
<td>Right vagal stimulation†</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>∆ HR (beats/min)</td>
<td>-21 ± 1*</td>
<td>-30 ± 7*</td>
<td>-23 ± 8*</td>
<td>-25 ± 7*</td>
</tr>
</tbody>
</table>

* Significantly different from no change (0.001 < P < 0.02).
† Ten seconds at 5 Hz, 1 mA, 1.24-msec pulse duration; n = 4 in each age group.

### Table 3 Lowest Test Dose of Catecholamine (μg/kg) Eliciting a Significant Change in Cardiovascular Function in Piglets of Different Postnatal Ages

<table>
<thead>
<tr>
<th>Function*</th>
<th>≤1 day</th>
<th>2-4 days</th>
<th>1 week</th>
<th>2 weeks</th>
<th>2.5-3 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Norepinephrine effects</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>↑ HR</td>
<td>0.25</td>
<td>0.10</td>
<td>0.10</td>
<td>0.05</td>
<td>Reflex bradycardia</td>
</tr>
<tr>
<td>↑ LV dP/dt max</td>
<td>0.25</td>
<td>0.25</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>↑ DBP</td>
<td>0.10</td>
<td>0.10</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>↑ Car R</td>
<td>0.25</td>
<td>0.25</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>↑ Fem R</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

| Isoproterenol effects |        |          |        |         |              |
| ↑ HR     | 0.10   | 0.05     | 0.05   | 0.05    | 0.05        |
| ↑ RV dP/dt max | 0.05   | 0.05     | 0.05   | 0.05    | 0.05        |
| ↑ DBP    | >0.10† | >0.10†   | 0.10   | 0.05    | 0.05        |
| ↑ Car R  | 0.10   | 0.10     | 0.05   | 0.05    | 0.05        |
| ↑ Fem R  | 0.10   | 0.10     | 0.05   | 0.05    | 0.05        |

* Increase (+) or decrease (−).
† Observed at 0.5 μg/kg but not 0.1 μg/kg (intermediate dose not tested). NS = changes not significant (P > 0.05).

Echolamines, but there were variations in magnitude of change to a given dose within each age group of piglets. The Fem R (Table 3) either increased or decreased after NE or E administration in piglets up to 2 weeks of age, such that mean changes were not statistically valid, and decreased (range, 1.0-1.3 PRU) in young piglets given at least 0.1 μg of ISP per kg. The mean decrease in Fem R in older animals given 0.05 μg of ISP per kg ranged from -1.9 ± 0.6 PRU (average 13%) at 1 week to -0.4 ± 0.1 (average 15%) at 2.5-3 months.

Relationships between Cardiovascular Responses and Catecholamine Dose

Linear regression analysis established a positive correlation between the five doses of NE or E and the simultaneous changes in HR (with the exception of older animals in which there was reflex bradycardia during the response to NE), in LV dP/dt max, and in AoP and DBP for each age group of piglets. Changes in other cardiovascular functions were not linearly related to NE or E dose. The HR effect of E is illustrated in Figure 3. The slope of this relationship between HR change and E dose was similar in all age groups of piglets.

Since only three doses of ISP had been tested, there were insufficient data for regression analysis. However, comparison of mean values for the change in a given parameter at the three different ISP doses established that changes in HR, RV dP/dt max, AoP, DBP, and Fem R were dose-dependent in each age group of animals.

### Age-Related Differences in Magnitude of Circulatory Responses to Catecholamines

The catecholamine dose chosen for this analysis (0.5 μg/kg) was one with which peripheral circulatory effects were obtained at all ages, and all piglets exhibited significant increases in HR and dP/dt max. Figure 4 presents bar diagrams of mean changes in DBP, Car R, and Fem R following administration of 0.5 μg/kg dose of each catecholamine in each age group of piglets.

Blood pressure responses are shown in the upper panels of Figure 4. The pressor effect of 0.5 μg of NE per kg, expressed as absolute or relative (percent) change, in piglets 2 weeks of age or younger was smaller than in piglets 2.5-3 months old. The same dose of E produced a fall in DBP only in the older animals. The depressor effect of this dose of
ISP, expressed as absolute or percent change, was smaller in piglets ≤1 week old than in older ones. The CAF increase in piglets given an 0.5 μg/kg dose of catecholamines was significant in all animals. The flow increase was accompanied by increased Car R in response to NE and decreased Car R in response to ISP, but variable changes in response to E (Fig. 4, middle panels). The percent change in resistance was greatest in the oldest animals.

The FF increased in young piglets given NE or E. However, there was variation in the direction and magnitude of change in Fem R in piglets given the 0.5 μg/kg dose of either NE or E. There were variable effects on FF in animals given 0.5 μg of ISP per kg, but Fem R decreased in all (Fig. 4, lower panels).

Confirmation of Presence of α- and β-Adrenergic Receptor Mechanisms in the Cardiovascular System of Newborn Piglets

The pressor and Car R effects of 0.5 μg of NE per kg were blocked (69–86%) within 10 minutes after administration of PT in the 25 piglets tested. The increase in HR and in RV dP/dt max produced by 0.1 μg of ISP per kg in piglets of all ages was blocked by PRP (73–92%). The depressor and vascular resistance effects of 0.1 μg of ISP per kg were blocked (87–93% and 69–80%, respectively) within 10 minutes after administration of PRP in the 22 piglets tested.

Discussion

Use of Pentobarbital Anesthesia

The nonphysiological condition of anesthesia with pentobarbital has been of general concern to physicians and nurses in the past.
investigators in developmental physiology (Assali et al., 1974; Boatman et al., 1965; Friedman, 1972; Gootman et al., 1972; Rudolph and Heyman, 1974; Woods et al., 1977). The argument on behalf of acute experiments in young animals under anesthesia has been supported convincingly by a comparison of data obtained in anesthetized fetal and maternal sheep with similar data from unanesthetized animals in which monitoring devices had been implanted (Assali et al., 1974). The depth of pentobarbital anesthesia achieved in our study was judged to be similar in each age group of piglets on the basis of the lid reflex. The particular problem posed in the present experiments was the assessment of age dependence of cardiovascular functions when increasing doses of pentobarbital were used to provide humane anesthesia in older animals. The basis for the much lower dose range of pentobarbital required to induce surgical anesthesia in the younger piglets is not clear. Since induction was equally prompt in all age groups, the comparatively slow processes of redistribution to fat, metabolic inactivation, or excretion cannot account for the differences in initial dose required. Low plasma albumin concentrations have been observed in young piglets (Ramirez et al., 1963); the increase from 0.8 to 1.63 and 2.36 g/100 ml between day of birth and first and second postnatal weeks, respectively, could result in somewhat higher proportions of unbound pentobarbital in the plasma of the younger animals. However, it seems likely that there are significant differences in the accessibility of the immature central nervous system (e.g., through the blood-brain barrier) and/or its pharmacological sensitivity to anesthetic agents, since a lower dose of halothane also was found to be sufficient to induce and maintain anesthesia in younger piglets (Crane et al., 1975).

Pentobarbital anesthesia has been reported to produce elevation of systemic arterial pressure in neonatal and adult swine (Hörnicke, 1966). However, we found that the values for aortic pressure (Table 1) were in the same range and increased with age, as had been observed previously in piglets and swine under local (Evans et al., 1963; Hörnicke, 1966), halothane (Buckley et al., 1976), or ketamine (Rowe and Arango, 1977) anesthesia. We did not find significant alterations in cardiovascular function after administration of small supplemental doses of pentobarbital at any age. Thus, despite the possible vulnerability of the developing cardiovascular and nervous systems to anesthetic agents, our catecholamine experiments should provide useful physiological information because: (1) initial and final control values of cardiovascular function did not differ appreciably, (2) catecholamine effects on blood pressure and heart rate were similar in decerebrate piglets before and after administration of the anesthetizing dose of pentobarbital, and (3) reflex responses to carotid occlusion were present. Furthermore, the results of catecholamine testing in piglets under pentobarbital anesthesia are applicable to questions arising from our earlier work on piglets under halothane anesthesia, since the catecholamine effects on aortic pressure and heart rate were similar in those animals tested under both types of anesthetic agent.

Postnatal Development of Cardiovascular Responses to Catecholamines

Catecholamine actions on blood pressure and heart rate have been examined in groups of fetal or neonatal mammals (Adams et al., 1958; Friedman, 1972; LeBlanc and Mount, 1968; Rudolph and Heymann, 1974), and some studies of age-dependent changes have been reported (Alexander et al., 1972; Boatman and Brody, 1967; Boatman et al., 1965; Gauthier et al., 1975; Hutchinson et al., 1962; Woods et al., 1977). However, few investigators have evaluated peripheral blood flow responses to catecholamines (Alexander et al., 1972; Boatman et al., 1965; Rudolph and Heyman, 1974). The cardiovascular responses in lambs (Alexander et al., 1972; Rudolph and Heyman, 1974; Woods et al., 1977), young dogs (Boatman and Brody, 1967; Boatman et al., 1965; Gauthier et al., 1975; Geis et al., 1975), and kittens (Hutchinson et al., 1962) indicate that there are differences in relative maturity of different mammals at birth. Our findings in piglets include: (1) age-related differences in catecholamine dose found to alter cardiovascular function, (2) age-related differences in magnitude of cardiovascular responses to a selected moderate dose of catecholamines, and (3) age-related differences in direction of heart rate and blood pressure changes after injections of NE and E, respectively. Thus, the piglet may be placed between lambs and young dogs on a scale of degree of maturity of adrenergic mechanisms at birth.

We have already established the pattern of responses to various experimental interventions in adult swine (Buckley et al., 1979; Gootman et al., 1978). Single injections of catecholamines in a wide range of doses in mature miniature swine led to immediate effects on the cardiovascular system resembling the responses observed in adults of other mammalian species. The following paragraphs summarize the postnatal change in response pattern that we have observed in piglets.

On day of birth, an increase in AoP occurred in response to doses of NE or E insufficient to elicit an increase in cardiac rate or contractility (Table 3). This implies a difference in threshold of cardiac and peripheral adrenergic effector systems and/or a difference in their rates of maturation. A similar discrepancy between threshold doses for aortic pressure and heart rate responses to these catecholamines also had been observed in young dogs (Boatman and Brody, 1967; Privitera et al., 1969). A positive chronotropic response could be elicited in some day-old piglets with the lowest dose of the more potent β-agonist, ISP, but not with that same dose of NE or E. The adrenergic effector system at
the sinoatrial node appears to be considerably less sensitive to its neurotransmitter in neonatal swine, and maturation of the chronotropic response to NE continues through the first week of life (Table 3). In contrast, chronotropic responses to catecholamines are very marked in the lamb heart during fetal life (Assali et al., 1974; Friedman, 1972; Rudolph and Heyman, 1974; Woods et al., 1977). The day-old piglets exhibited a marked positive inotropic response to the low dose of ISP (Table 3), which is in keeping with the reported presence of an active cardiac adenylate cyclase system at birth (Mersmann et al., 1977). The dose of ISP sufficient to increase cardiac contractility was not sufficient to lower aortic pressure, and a low dose of E that was depressor for older piglets was pressor at birth. These observations suggest that vascular β-adrenergic mechanisms are poorly developed in swine at birth, in contrast to the cardiac inotropic mechanism.

The carotid circulation on day of birth contains an active adrenergic effector system since carotid resistance could be increased by NE and decreased by ISP. In contrast, the femoral circulation exhibited variable changes in resistance when catecholamines were injected systemically, with the exception of the higher dose of ISP (Fig. 4). Asynchronous development of adrenergic and local myogenic or metabolic mechanisms involved in regulation of blood flow through the femoral circulation could account for these observations.

When piglets are between 2 and 4 days of age, cardiac mechanisms have become more reactive, although vascular adrenergic mechanisms appear to be the same as at birth (Table 3). There was still no depressor response to a low dose of E or ISP at this age, indicating that vascular β-adrenergic mechanisms are still underdeveloped.

At the end of the 1st postnatal week, cardiac adrenergic mechanisms are still more reactive (Table 3). Depressor effects of the lowest dose of ISP were observed in all piglets, and the depressor effect of E occurred in some, indicating a further maturation of the β-adrenergic receptor mechanisms in the peripheral vasculature. The femoral circulation in week-old piglets is one site of such maturation, since femoral resistance decreased in all week-old animals given the lowest dose of ISP.

By the 2nd postnatal week, most of the cardiovascular functions examined in this study were altered qualitatively (but not always quantitatively) by the selected catecholamines in the same manner as in the 2.5 to 3-month-old animals (Figs. 2 and 4) and mature swine (Buckley et al., 1979). The two notable exceptions, variability of femoral circulatory responses and absence of reflex bradycardia during the pressor response to NE in piglets, are discussed separately below. Although NE-induced changes in aortic pressure were independent of age in piglets up to 2 weeks old, the increase at 2 weeks was smaller than at 2.5–3 months (Fig. 2, left) and significantly smaller than in mature swine (Buckley et al., 1979). In contrast, blood pressure effects of 0.2 µg/kg doses of NE were of the same magnitude in unanesthetized neonatal lambs as in ewes (Woods et al., 1977). However, 1- to 2-week-old dogs were less responsive than adult dogs when given NE in doses ranging from 0.01 to 1.0 µg/kg (Privitera et al., 1969). It appears that adrenergic inotropic and vasoconstrictor mechanisms, although present at birth, undergo detectable postnatal maturation in piglets and young dogs. In their analysis of the maturation of β-adrenergic mechanisms in the cardiovascular system, other investigators (Friedman, 1972; Rudolph and Heymann, 1974; Woods et al., 1977) have been examining cardiac but not regional blood flow responses to ISP. The relatively slow maturation of the vascular β-adrenergic receptors is still continuing beyond the 2nd postnatal week in swine, as is clear from our results.

The directional variation in femoral resistance in young piglets given NE or E implies that the adrenergic vasoconstrictor mechanisms are still maturing after the 2nd postnatal week. For this conclusion to be valid, it is important that changes in femoral flow during the pressor response to NE or E not be passively dependent upon changes in aortic pressure or CO. It should be noted that the femoral circulation undergoes vasoconstriction in response to 1–2 µg NE in newborn dogs (Boatman et al., 1965) and lambs (Alexander et al., 1972) and that iliac vessels isolated from dogs on the day of birth are very reactive to NE (Cox et al., 1976). Since a NE dose of 0.1 µg/kg did increase femoral resistance in mature swine (Gootman et al., 1978), and the increase could be blocked by PT administration, we concluded that there is an active α-adrenergic mechanism in the femoral circulation of this mammal. However, it is not functioning well during early postnatal life. Our results do not differentiate between immaturity of the vasoconstrictor mechanism and the possible presence of vasodilator mechanisms. Although a β-adrenergic vasodilator mechanism was relatively insensitive to low doses of ISP in young piglets, it could be demonstrated in the femoral circulation of all piglets given 0.5 µg of ISP per kg (Fig. 4, right panel). This finding suggests that adrenergic vasodilator mechanisms in the femoral circulation are functionally immature in young piglets.

Reflex bradycardia did not occur during the pressor effect of NE in piglets, whether they were decerebrate or under anesthesia with pentobarbital (see also Adams et al., 1958), halothane (Buckley et al., 1976), or locally injected xylocaine (Gootman et al., 1979). Our findings continue to emphasize a species difference between newborn piglets and lambs, since the latter exhibit reflex bradycardia during the pressor effect of a 0.2 µg/kg dose of NE (Woods et al., 1977). The adequacy of the stimulus...
detected by the baroreceptors in our piglets is demonstrated by the following observations. A 10 mm Hg increase in AoP, which was accompanied by reflex bradycardia in mature swine given 0.05 μg of NE per kg (Gootman et al., 1978), was also attained in 1- and 2-week-old piglets given 0.1 μg of NE per kg, but again without the appearance of bradycardia; a 24 mm Hg increase in AoP in mature swine given 0.1 μg of NE per kg (Gootman et al., 1978) was attained in many piglets given 0.5 or 1.0 μg of NE per kg, but still without the occurrence of bradycardia in any piglet. Thus, pressure changes adequate to elicit reflex bradycardia in mature swine were not adequate in piglets. However, the baroreceptors and afferent pathway of the reflex were functioning because aortic pressure did increase, again without heart rate change, during carotid occlusion tests carried out as a qualitative check on cardiovascular depression by pentobarbital. In our earlier studies on effects of afferent and direct stimulation of the cardiac end of the right vagus nerve in piglets of differing ages (Table 2) provided the evidence that the cardiac efferent vagal pathway of the baroreceptor reflex was capable of function in young piglets. Alteration in cardiac function following these procedures implies the presence of tonic vagal activity and/or disinhibition of sympathetic autonomic activity and is consonant with observations of early maturation of cardiac vagal innervation in other mammals (Assali et al., 1974; Rudolph and Heyman, 1974; Woods et al., 1977). The results of our experiments suggest that the absence of reflex bradycardia during pressor effects of NE or bilateral carotid occlusion is a consequence of immaturity of central cardiovascular regulation.

In conclusion, we can now interpret the results of our earlier studies on effects of afferent and direct stimulation of the cardiovascular regulatory centers (Buckley et al., 1976; Gootman et al., 1972; Reddy et al., 1974) as dependent on peripheral as well as central autonomic immaturity in piglets up to 2 weeks of age. Asynchrony of development of peripheral effectors for cardiac and vascular responses may contribute to the absence or variability of the cardiac component of responses to afferent or central stimulation. Asynchrony of development of peripheral vasoconstrictor and vasodilator mechanisms may contribute to the difficulty in eliciting depressor responses during afferent stimulation.

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Increased Sheep Lung Vascular Permeability Caused by Escherichia coli Endotoxin

KENNETH L. BRIGHAM, RONALD E. BOWERS, AND JAMES HAYNES

SUMMARY We infused Escherichia coli endotoxin, 0.07-1.33 μg/kg, intravenously into chronically instrumented unanesthetized sheep and measured pulmonary arterial and left atrial pressures, lung lymph flow, lymph and blood plasma protein concentrations, and arterial blood gases. Endotoxin caused a biphasic reaction: an early phase of pulmonary hypertension and a long late phase of steady state increased pulmonary vascular permeability during which pulmonary arterial and left atrial pressures were not increased significantly and lung lymph flow was 5 times the baseline value. Lymph: plasma total protein concentration ratio during the late phase (0.76 ± 0.04) was significantly higher than during baseline (0.66 ± 0.03). The lymph response was reproducible. Lung lymph clearance of endogenous proteins with molecular radii (r) 35.5 to 96 Å was increased during the steady state phase of the reaction, but, as during baseline, clearance decreased as r increased. The endotoxin reaction was similar to the reaction to infusing whole Pseudomonas bacteria, except that endotoxin had less effect on pressures during the steady state response and caused a relatively larger increase in lymph clearance of large proteins. We conclude that E. coli endotoxin in sheep causes a long period of increased lung vascular permeability and may have a greater effect on large solute pathways across microvessels than do Pseudomonas bacteria. Circ Res 43: 282-287, 1979

GRAM-NEGATIVE sepsis is one cause of noncardiac (primary) pulmonary edema in humans (Robin et al., 1972, 1973). Changes in the lung similar to those in the human disease can be produced in animals by infusing E. coli endotoxin (Snell and Ramsey, 1969; Reeves et al., 1972). Although, by inference, endotoxin seems to increase lung microvascular permeability, there is little specific information about effects on permeability (Chien et al., 1964). We showed earlier that infusing whole Pseudomonas bacteria intravenously into unanesthetized sheep caused transient marked pulmonary hypertension followed by a long period of high flow of protein-rich lung lymph with stable pulmonary vascular pressures, indicating high exchanging vessel permeability (Brigham et al., 1974; Brigham et al., 1976b). Now we have infused E. coli endotoxin into unanesthetized sheep and produced a similar biphasic reaction: transient pulmonary hypertension followed by a long period of high permeability (i.e., high flow of protein-rich lung lymph with stable vascular pressures). The endotoxin response was reproducible. The effects of endotoxin and Pseudomonas on permeability were different in that endotoxin caused lymph clearance of large proteins to increase more than did Pseudomonas. We conclude that intravenous Escherichia coli endotoxin in sheep causes a lung vascular reaction similar to that caused by whole Pseudomonas bacteria but that, during the high permeability caused by endotoxin, exchanging vessels sieved larger proteins less effectively than after Pseudomonas. This may imply that endotoxin has a greater effect on large solute pathways ("large pores"[Blake and Staub, 1976; Harris et al., 1976]) in lung microvessels.
Age-related cardiovascular effects of catecholamines in anesthetized piglets.
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