Accelerated Development of Salivary Glands of Early Postnatal Rats Following Isoproterenol.* (28029)

CHARLOTTE A. SCHNEYER AND JOHN M. SHACKLEFORD
Departments of Physiology and Anatomy, University of Alabama School of Dentistry and Medical College, Birmingham, Ala.

Salivary gland enlargement has recently been observed in adult rat following chronic administration of the sympathetic drug, isoproterenol(1-3), or as a result of stimulation of the sympathetic innervation to the glands (4). This enlargement results primarily from cellular hypertrophy(2,3) but hyperplasia has been observed also(1,3). Furthermore, loss of cellular differentiation, although reversible, is also apparent(3). Thus, although hypertrophy, probably resulting from increased activity, is the primary response, the loss of differentiation and occurrence of hyperplasia suggest the possibility that sympathetic agents normally influence mitotic activity and cellular differentiation. In rat, salivary glands continue differentiation for several weeks following birth(5,6) and hence may provide a useful system for investigation of effects of sympathetic agents on the differentiation process. Therefore, as a first step, the effects of chronic administration of isoproterenol on differentiation in salivary glands of very young (1-4 days at initiation of treatment) rats were investigated.

Methods. Litters of Long-Evans rats were divided at birth into control and experimental groups consisting of, as far as possible, a similar number of males and females. To the experimental groups, 1 to 4 days after birth, the sympathetic agent, isoproterenol, was administered intraperitoneally twice daily (1.5 mg/dose) for a selected number of days. Control animals were either untreated or injected with saline. At selected intervals a control and experimental animal of the same sex from each group were sacrificed, and fragments of the salivary glands were fixed in Bouin's solution or buffered formalin. Tissues were stained with hematoxylin and eosin (Bouin's fixative), or were subjected to procedures designed to reveal histochemical composition of tissues. These included the periodic acid Schiff (PAS reaction), and the p-hydrazinobenzenesulfonic acid (PBSA) method and the alcian blue method.

Results. Chronic administration, twice daily, of 1.5 mg of isoproterenol to rats 1 to 4 days of age at time of first injection had little effect on body size and weight for the first 7 days of treatment. Thereafter, retardation of growth became evident, but only after 15 days of drug administration was the change significant (15-20%). By 35 days of age, the treated animals were only one-half the size of the controls when litter-mates were compared. Injection of saline solution twice daily for similar periods had no appreciable effect on body growth. Results of a typical experiment on the effect of isoproterenol on body size are shown in Table I. During the period of isoproterenol treatment marked increase in size of selected organs, notably the parotid and submaxillary glands, occurred, as shown, also, in Table I. This increase in organ size became evident before total body growth became affected by isoproterenol treatment and remained prominent even after body growth had appreciably slowed. Sublingual salivary gland did not respond in the same manner as parotid and submaxillary glands; no accelerated increase in the size of the sublingual gland in response to isoproterenol was observed at any stage here examined. In other experiments where heart size was measured after periods of isoproterenol treatment, an early small increase was observed but this became obscured after 2-3 weeks of treatment, when body size was markedly affected. Nonetheless, when heart size was related to body size at each stage, it became clear that the ratio of heart size to body size was larger in the experimental groups than in the controls. Other organs, including adrenal gland, spleen, kidney and testis, after 35 days of treatment, were not increased in size in re-

* This study was aided in part by grants from Nat. Inst. Health.

TABLE I. Effects of Chronic Administration of Isoproterenol on Animal and Salivary Gland Size of Early Postnatal Rats.

Animal Group*	Days on ISO	Animal size (g)		Size (mg) parotid		Size (mg) submaxillary		Size (mg) sublingual	
		EXP	CON	EXP	CON	EXP	CON	EXP	CON
E-C 7	4	12.5	12.5	(3)	(2)†	18	15	3	5
E-C 10	7	15	16	6	4	30	23	5	5
E-C 14	11	21	24	21	15	33	30	7	7
E-C 16	13	27	28.5	38	12	56	35	7.5	7
E-C 18	15	26	31.5	54	38	70	40	8.5	9
E-C 21	18	37	45	88	50	82	53	12.5	13.5
E-C 36	35	74	134	368	200	272	110	25	29

* All animals except E-C 36 are from the same litter; experimentals (E) were placed on the isoproterenol regimen when rats were 3 days of age; controls (C) were not placed on this regimen; numbers following E-C designate age of animal pair (days) at time of sacrifice.

† Parentheses indicate that values are approximate.

TABLE II. Effects of Chronic Administration of Isoproterenol on Heart Size as Compared with Body Size.

Animal size		Age	Days on ISO	Heart size		Heart size/ Body size	
CON	EXP			CON	EXP	CON	EXP
(g)		(days)		(mg)		(mg/g)	
24	22.5	14	10	112	125	4.7	5.5
27	26	15	14	122	152	4.5	5.9
30	32	18	14	180	204	6.0	6.4
86	67	28	27	349	308	4.1	4.5
134	74	36	35	498	315	3.7	4.3

Litter-mates used; representative animals, pairs from several experiments. Age at which ISO regimen initiated = (Age − days on ISO).

lation to controls, either absolutely or in relation to body weight, but like body weight, were approximately one-half the size found for controls. Effects of isoproterenol on heart size are shown by the representative data in Table II.

Accelerated morphological differentiation was observed in parotid and submaxillary glands of early postnatal rats subjected to the isoproterenol regimen, and this could be separated from the hypertrophy effects. These differentiation effects were especially pronounced in parotid gland. Photomicrographs of representative sections of parotid glands, from the same experimental series referred to in Table I, are shown in Fig. 1 and 2; in both cases, controls and experimentals are designated C and E, respectively, and the age of the animal, in days, follows these designations. Thus, it is clear from Fig. 1 (C-E 7) that after only 4 days of treatment with isoproterenol, the acini of the experimental gland are advanced in development when compared with that of the litter-mate control. In the control parotid, almost all of the cells are undifferentiated, with no definitive acini and only a few striated ducts; in the experimental, on the other hand, many cells are of pyramidal shape and therefore already resemble acinar cells. There is also a greater concentration of striated ducts. By 7 days of isoproterenol treatment (E-10), many fully developed parotid acini are observed, with characteristic oval-shaped, basally placed nuclei, while the control gland, C-10, is still composed mainly of undifferentiated cells, with only a few pyramidal cells evident. Again, more striated ducts are observed in the experimental than in control. At the end of 11 days of isoproterenol treatment, acini at least as large as those of the control adult parotid glands are seen, while the litter-mate control parotid continues to show, primarily, undifferentiated small cells, with only a few presumptive acini apparent (Fig. 1, C-E 14). It is not until the control reaches 16 days of

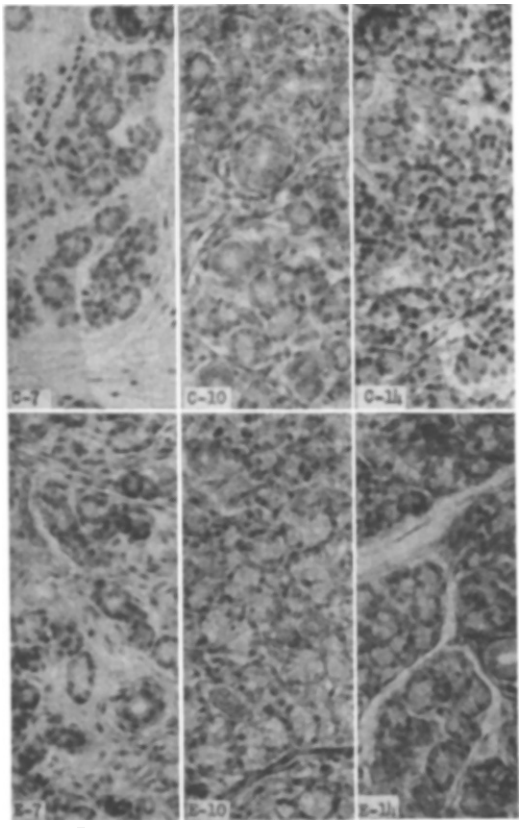

FIG. 1. C 7-14: Parotid glands from control animals. Numbers indicate age of animals in days. Few if any acini are visible in this series; mostly undifferentiated cells appear. E 7-14: Parotid glands from experimental animals, litter-mates of C 7-14. Definitive acini are visible as early as E-7.

age that some well developed acini are seen, and even here, most of the cells are still undifferentiated. Not only are more acini prominent in the experimentals at this stage but they are larger in size than those of a normal adult parotid gland and already show a vacuolated foamy appearance characteristic of glands of adults treated with isoproterenol (3). This resembles somewhat the appearance of a mucus-secreting gland (Fig. 2, C-E 16). With further duration of isoproterenol treatment, enlargement of acinar cells continues but no enlargement of cells of the striated ducts occurs. (Even in a 36 day old control animal, some undifferentiated cells and some mitotic figures are seen while the experimental of the same age (isoproterenol, 35 days) shows only very much enlarged mucus-appearing acini and normal-sized cells in compressed striated ducts (C-E 36).)

Similar changes are observed in submaxillary glands but since development of the parotid proceeds more slowly, normally, than development of submaxillary gland, the effects in the submaxillary are not so striking as in parotid. (After 4 days of treatment with isoproterenol, the submaxillary gland shows acini more fully developed than those of the control gland; by 7 days of treatment, the acini are beginning to resemble mucous cells, and by 11 days, the acini are not only well developed but exceed in size those of the submaxillary of the adult animal. In the control, the acini are not fully developed

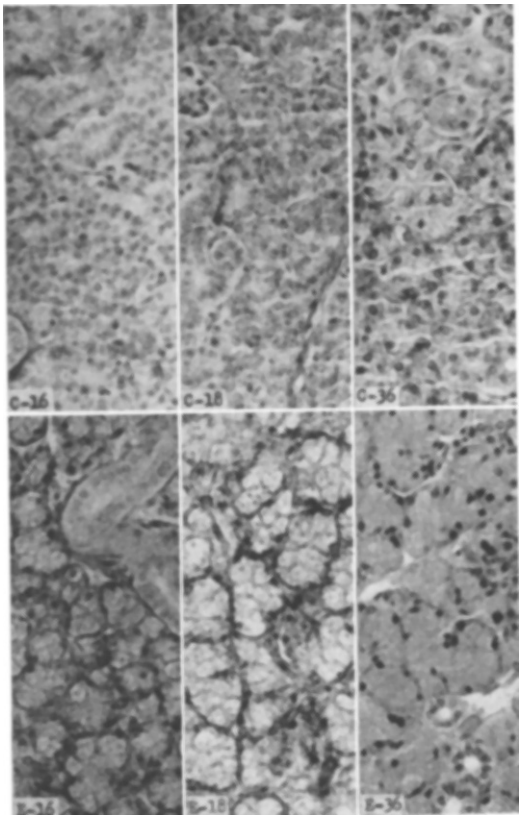

FIG. 2. C 16-36: Parotid glands from control animals 16, 18, and 36 days of age, respectively. Gland is still composed primarily of undifferentiated cells, and not until C-36, are numerous acini seen. E 16-36: Parotid glands from experimental animals, litter-mates of C 16-36. In each instance, acini are not only prominent but greatly enlarged, even when compared with parotid acini from adult animals.

at this stage and do not become so for some time.)

Glands were examined for carbohydrate, as demonstrated by the p-hydrazinobenzenesulfonic acid reaction. Parotid glands of 7 day old control and isoproterenol-treated animals did not differ histochemically and showed the color reaction characteristic of low concentration of carbohydrate, usually found in the serous parotid gland at this stage. After 7 days of treatment with isoproterenol (10 day old animals), however, a marked histochemical difference is noted: the PBSA reaction of the experimental parotid gland indicates a marked increase in tissue concentration of carbohydrate, suggestive of chemical modification away from serous, and toward a mucous, cell type.

Discussion. An effect of isoproterenol on cell size of salivary glands of adult animals is now well established(1,2,3). The present investigation shows that isoproterenol administered to young postnatal rats, while effecting an increase in cell size here also, accelerates the rate of differentiation as well. It has been noted that morphological differentiation is accelerated before the increase in cell size is detected. Differentiation generally proceeds on a biochemical as well as a morphological level, and administration of isoproterenol appears to affect both aspects of differentiation. This effect is most marked in the parotid gland. The influence on morphological differentiation takes the form of early formation of definitive acini and an increase in number of striated ducts, while the influence on biochemical differentiation, which has been noted, consists of alteration toward a more mucus-like gland, as revealed by PBSA response.

In the young animals used in this investigation, isoproterenol treatment results in an increase in size of similar extent in parotid and submaxillary glands, although in adults treated with isoproterenol, the increase in size of the parotid gland far exceeds that for submaxillary(3). This difference is probably at least partly a reflection of the fact that granular tubules are not present in the submaxillary gland until the animal reaches 6 weeks of age(5) and are not present in parotid gland at any age, and further, that cell-size of granular tubules has not appeared to be much increased by isoproterenol. Hypertrophy is due primarily to increase in size of acinar or presumptive acinar elements. These, in the young submaxillary, are not diluted by the presence of granular tubules, but in the adult submaxillary are. Although the size of granular tubule cells has not been seen to increase during isoproterenol treatment while these cells can still be clearly distinguished (4 to 5 days of treatment), the precise fate of the granular tubule cells with prolonged isoproterenol treatment is difficult to determine, since then they can no longer be distinguished from acini(3). Whether there has been transformation into some other element, or loss of differentiation (granules disappear, tubules become compressed, and response to histological stains may not differ from that of acini) with accompanying loss of specific identity, cannot be decided at the present time.

The increase in heart size, while small, generally confirms observations previously made on the heart of adult female rats(3), and suggests that the effect involves at least one other organ which, under physiological conditions, responds to sympathetic stimulation by activity.

A further effect, observed incidentally, is that administration of isoproterenol to the young animals caused eye-lid opening two to three days in advance of controls. All of the effects noted have, of course, been induced by pharmacologic, not physiologic doses of isoproterenol. Further refinement of approach is required to delineate more clearly the normal role of sympathetic amines in growth and development.

Summary. Chronic administration of the sympathetic amine isoproterenol to early postnatal rats (1-4 days old at time of first injection) for selected periods results in marked increases in the size of the submaxillary and parotid glands, and to a less extent, in the size of the heart. The sublingual salivary gland is unaffected. Other organs are reduced in size, especially after 3 to 5 weeks, when a retardation in body size and weight also becomes pronounced. A clear effect on

differentiation of the parotid and submaxillary gland has been observed with isoproterenol treatment. The effect is most marked in parotid, where acini develop much earlier than in the litter-mate controls and striated ducts earlier become more abundant. The effects of isoproterenol on differentiation are separable from the influence of the drug on cell size.

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Received September 7, 1962. P.S.E.B.M., 1963, v112.

Effect of Drug Infusion on the Splanchnic Circulation. I. Angiotensin Infusion in Normal and Cirrhotic Subjects. (28030)

L. CHIANDUSSI, A. VACCARINO, F. GRECO, F. MURATORI, L. CESANO, D. INDOVINA
(Introduced by S. Sherlock)
Institute of Medical Pathology, University of Turin, Turin, Italy

The physiology and pharmacology of angiotensin have recently been reviewed(1). Although there is considerable literature on its effect on cardiac output, blood pressure and peripheral resistances, there are few data on the effects of angiotensin on regional hemodynamics and, particularly, on splanchnic circulation. Abell and Page(2) noted a narrowing of the mesenteric vessels after angiotensin. Barer(3) found mesenteric blood flow to be reduced in the cat after single injections of 0.2 to 0.4 γ. Mandel and Sapirstein(4) could not observe in rats, under infusion of angiotensin at different rates, any significant variation in splenic and intestinal blood flow.

The present communication concerns the effects of intravenous infusion of angiotensin on splanchnic hemodynamics in normal and cirrhotic subjects.

Material, methods and procedure. The subjects were divided into 2 groups. Group I consisted of 8 subjects without liver and cardiovascular disease; Group II consisted of 7 patients with liver cirrhosis and portal hypertension.

After catheterization of a main right hepatic vein, hepatic blood flow (EHBF) was measured by a constant infusion of Indocyanine Green, following the Fick principle (5). At least 4 pairs of arterial and hepatic venous blood samples were obtained for estimation of basal hepatic blood flow.

Mean arterial pressure (MAP) was frequently recorded using a Rangoni-Battaglia electromanometer; the tracings were electrically damped.

The intrasplenic pressure (ISP) and the wedged and free hepatic vein pressures (WHVP and FHVP) were, alternatively, simultaneously recorded by a couple of Rangoni-Battaglia electromanometers with zero reference point 5 cm below the sternal angle. They were repeatedly recorded before and during the angiotensin infusion.

Portal venous pressure (PVP) was calculated from the formula:

$$\text{PVP (mm Hg)} = \frac{\text{ISP} + \text{WHVP}}{2}$$

or was assumed to equal the WHVP.

Splanchnic resistances (SR) were calculated from the formula:

$$\text{SR (dynes/sec/cm}^{-5}) = \frac{(\text{MAP} - \text{PVP}) \times 80}{\text{EHBF (l/min)}} (6).$$

Cardiac output (CO) was measured by an indicator dilution technic, using 10 μc of RISA. It was measured before and during the angiotensin infusion.

The rate of intravenous infusion of angio-