Modification of Irradiation Effects on Rat Parotid by Chronic Pretreatment with Isoproterenol* (33961)

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Some catecholamines such as epinephrine and dopamine have been shown to provide protection against effects of irradiation when administered prior to the radiation dose (1, 2). However, norepinephrine does not have this protective property (3). The present study was therefore undertaken to test another catecholamine for radioprotective properties. This catecholamine, isoproterenol, is known to act primarily on β adrenergic receptors (4). In salivary gland a single injection of isoproterenol causes increased physiological activity by eliciting profuse salivation (5), and given over longer periods, it also promotes glandular growth that involves hyperplasia, hypertrophy, and polyploidy (5–10). This agent thus has the potential for modifying effects of irradiation in a number of diverse ways. In the present study, isoproterenol exerted a protective effect against X-irradiation of parotid gland when the drug is administered during a specific and limited period preceding the irradiation dose. This period also corresponds to the time during administration when mitosis rather than hypertrophy is prominent in the growth response to the catecholamine.

Materials and Methods. Female Fischer rats, 120–150 days old and weighing 146–200 g, were used in the experiments. Animals were maintained on a diet of lab chow except for 18-hr periods preceding irradiation and sacrifice; water was available at all times. X-Radiation was delivered (during a single exposure of 10-min duration) to the right parotid gland by a 160 kVp, 20 mA, Picker X-ray therapy machine; physical factors were as follows: 70 kVp, 15 mA, no filtration, half-value layer 0.7 mm Al, target-skin distance 19 cm, field size 5.3 cm^2, 420 R/min. Rats were anesthetized by ip injection of Seconal (20 mg/kg) and were then placed in an acrylic, lead-shielded rat holder, designed to protect all vital structures except those in the line of radiation beam. With the rat in the holder, a port keyed to receive a 2.5-cm diameter X-ray cone was located over the lateral surface of the right parotid gland. Dosage was 3000 R; this delivered 2878 rads to the midpoint of the gland at a depth of 3 mm. Such a dose was chosen since a marked reduction in gland weight and alteration of histological appearance was produced by it (11). Skin dose was calculated to be 3980 rads, and dose to left parotid, 35 rads. A Con-Rad LiF-Teflon rod dosimeter, at selected tissue depths, was also used to record dose. The calculated and empirical values were very similar.

Isoproterenol–HCl (1-3, 4-dihydroxyphenyl) 2-isopropylaminoethanol hydrochloride) (Winthrop Laboratories, New York, N. Y.) was administered ip twice daily (6 mg/injection), except in the acute experiment where only a single 6-mg dose was administered 1.5 hr before irradiation. In the chronic experiments, the last injection of (isoproterenol) ISO was given 18 hr preceding irradiation. The parotid glands were removed approximately 96 hr (4 days) following irradiation; maximal decrease in gland weight was previously shown to occur during this interval (11). Each gland was then weighed, and a sample was placed in formalin or Bouin's for subsequent histological examination. Tissues were stained with hematoxylin and eosin before microscopic examination.

Results. Mean weights of right and left parotid glands of untreated rats do not differ

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TABLE I. Effect of Irradiation on Gland Weight of Isoproterenol-Treated Rat Parotid.[a]

Treatment	No. of animals	Gland wt (mg)[b]		Decrease (%)
		IRR	Control	
None	10		147 ± 7[c]	
IRR[d]	8	95 ± 7	137 ± 2	31[f]
ISO, 1.5 hr + IRR[e]	5	118 ± 10	159 ± 10	26[f]
24 hr + IRR	3	246 ± 17	302 ± 9	19[f]
48 hr + IRR	4	252 ± 20	255 ± 22	1
72 hr + IRR	8	225 ± 14	238 ± 14	5
240 hr + IRR	5	227 ± 28	329 ± 23	31[f]
IRR–ISO, 96 hr	4	302 ± 43	423 ± 22	29[f]

[a] Means ± SE.

[b] Irradiated (IRR) glands and contralateral control glands; all animals were killed 4-days post-irradiation, and fasted for 18 hr preceding irradiation and sacrifice.

[c] Mean weight of right and left glands of the nonirradiated controls did not differ significantly from each other (R = 152 ± 10.8, L = 141 ± 7.2 for gland wt).

[d] Dose: 3000 R to right parotid; left parotid received less than 35 R, in each condition where irradiation was given.

[e] Dose: 6 mg of ISO ip 1.5 hr prior to irradiation. All others received ISO (6 mg ip twice daily) for period (hr) stated. In all cases, sequence of treatment is indicated.

[f] Differences between controls and experimentals are statistically significant ($p < .001$).

significantly ($p > .01$) from each other (Table I). Four days after a single dose of 2878 rads, rat parotid glands exhibited a 30–35% decrease in weight when compared with the nonirradiated contralateral glands or control glands of nonirradiated animals (Table I). However, when isoproterenol was administered twice daily for 2 or 3 days prior to irradiation, the expected decrease in weight of the irradiated gland did not occur. As shown by the data in Table I, the weights of irradiated and nonirradiated glands of such ISO-treated rats were the same. On the other hand, administration of ISO for periods less (1.5 hr or 1 day) or greater (10 days) than 2–3 days resulted in no, or only slight, protection from the effects of irradiation. Thus, when ISO was administered twice daily for 10 days before irradiation, or only administered as a single stimulatory dose 1.5 hr before irradiation, the irradiated gland showed the usual radiation-induced decrease in size from that of the nonirradiated control (approx 30%). One day of ISO preceding irradiation resulted in slight protection.

Finally, it may be noted that administration of ISO for 3 days after irradiation (with no ISO preceding irradiation) did not facilitate recovery from irradiation: size of the irradiated glands was about 30% less than that of the nonirradiated glands.

Histological examination of the irradiated glands revealed changes that were in most respects similar to those previously reported for irradiated salivary glands (12–15). These included degenerative changes of the nuclei (pyknosis, fragmentation), and vacuolization and atrophy of acinar cells. It may be noted that areas showing irradiation-induced changes alternated with normal-appearing regions. No discernible effects were noted in the ductal elements (Fig. 1A, B).

When ISO was administered for 2 or 3 days prior to irradiation, the irradiated glands were indistinguishable from contralateral controls in histological appearance. In both cases, markedly enlarged acinar cells were observed, while little or no degenerative changes attributable to irradiation could be seen (Fig. 1C, D). In animals which had received ISO for 1½ hrs, 1 or 10 days prior to irradiation, the effects of irradiation were discernible and were, in some cases, as pronounced as those observed in glands untreated

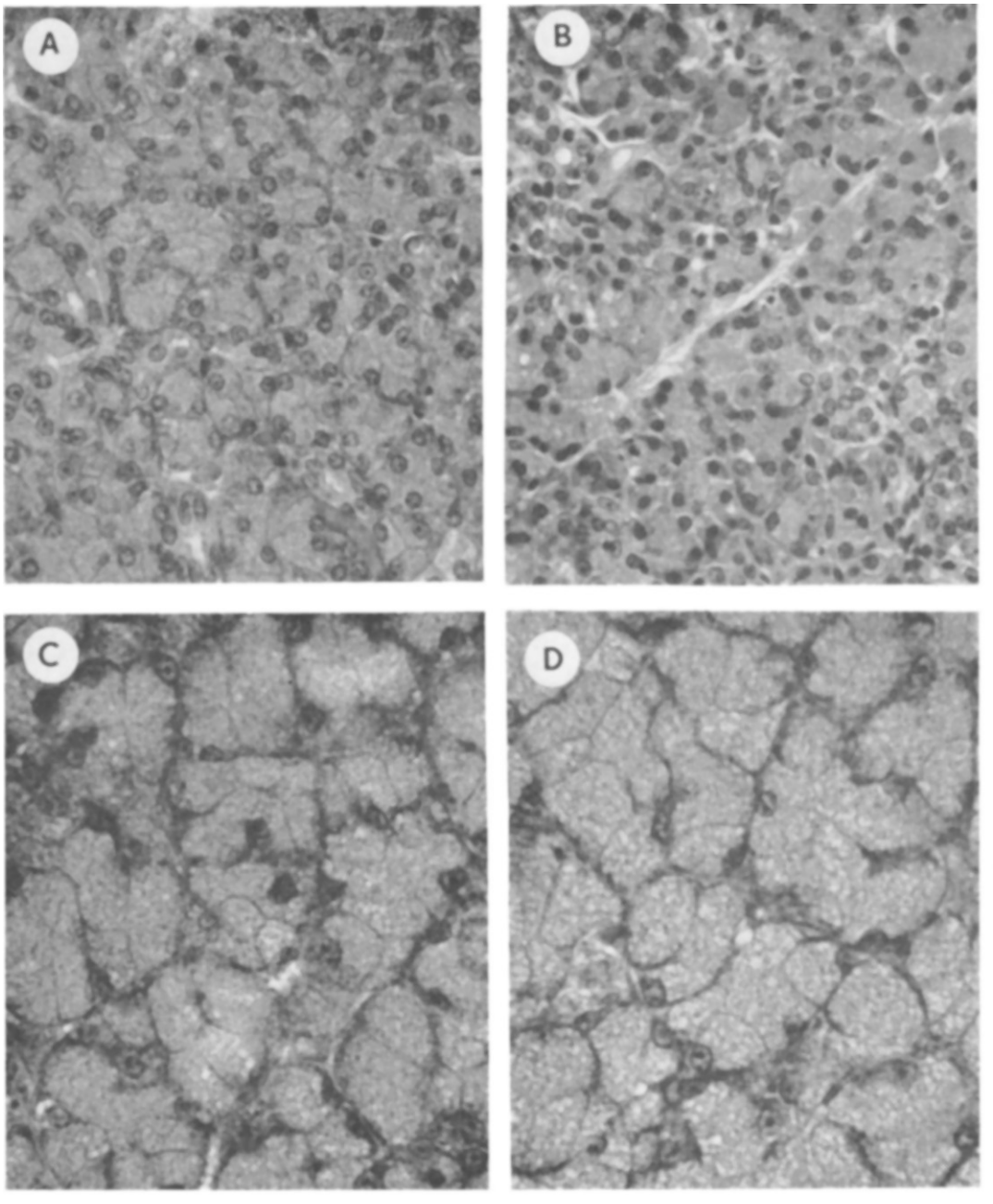

FIG. 1A. Nonirradiated parotid gland of rat, and (B) contralateral irradiated paratid 4 days after a single exposure to 3000 R: (C) nonirradiated parotid, and (D) irradiated parotid of rat maintained on a regimen of twice daily injections (6 mg) of isoproterenol for 3 days prior to irradiation. Note fragmented and pyknotic nuclei in B, and decrease in acinar size from that of control (A). Acinar cells in (C) and (D) are distinguishable from each other in appearance; both show characteristic ISO-induced enlargement but irradiation-induced changes are not evident; ×431.

with ISO. After 10 days of ISO, however, the cells were so enlarged that it was often difficult to assess changes in structure.

Discussion. The administration of isoproterenol to rats for 2 or 3 days prior to irradiation of the parotid gland apparently protects the gland from the weight loss and cellular damage usually observed with irradiation alone (11–14). Periods less than this provided only slight protection, while administration of ISO for 10 days provided no protection at all. Furthermore, a single stimulatory dose did not protect the gland from irradiation effects. Thus, the protective effects of this agent do not appear related either to increased secretory activity induced by stimulation of the gland (5, 6, 15) or to the mere presence of the catecholamine (1–3). It does appear to be related to gland enlargement induced by the ISO (5), and in fact, to a specific phase of the enlargement. When ISO is administered over periods extending from 1 to 10 days, a gland enlargement occurs that is characterized by an early phase (2–4 days) of hyperplasia that is accompanied and superseded by a later phase (8–10 days) of hypertrophy. During the early phase, mitotic activity (7, 9, 16) and DNA (9, 16) increase markedly, and polyploid as well as diploid cells are produced (7, 8). It thus appears that protection against irradiation occurs during the period when hyperplasia and polyploidy are the predominant cellular events. By 10 days, when mitotic activity (leading to diploid or polyploid cells) is virtually indiscernible, no protection is observed. The fact that the period characterized by increased mitotic activity affords protection against irradiation appears at first in contradiction to other reported observations. It is well known for example that radiosensitivity of cells that show high mitotic activity is greater than that of cells which exhibit a lower degree of mitotic activity (17, 18). However, the situation here induced by ISO is unusual in that much of the increased mitotic activity leads to polyploidy (7). Therefore, the polyploidy per se or changes in the chromosomes or mitotic apparatus, induced by the ISO, may have some role in providing protection against the irradiation. This remains to be elucidated.

Finally it may be mentioned that although size of the glands was appreciably increased when the isoproterenol regimen was employed, the increased depth of the tissue was probably not the factor involved in providing the protection against the irradiation. Gland size is increased only 1.5–2 times (7) at 2–3 days when protection from irradiation is observed; at 10 days, when there is no protection, there is even a greater increase in gland size (5 times normal) (7).

Summary. Twice daily administration of the sympathetic amine, isoproterenol, for 2 or 3 days prior to irradiation (3000 R) of rat parotid glands afforded protection from the effects of irradiation and the usually observed changes in size and structure of the gland did not occur. When the amine was administered for shorter periods (1.5 hr) or much longer periods (10 days) no protection against the effects of irradiation was observed, and under these conditions, the usual radiation-induced effects (*i.e.,* reduction in size of gland and of acinar cells, and pyknosis and fragmentation of nuclei) were seen.

The protective effects of isoproterenol are not considered, from this work, to be related either to increased secretory activity of the gland or to the presence of this catecholamine. The ISO also causes a gland enlargement characterized by an early phase (following initiation of the ISO regimen) of increased mitosis (2–4 days) with diploid and polyploid cells produced, and a later phase (without discernible mitosis) of hypertrophy (10 days ISO). The protection afforded by ISO therefore appears related to the gland enlargement induced by the ISO, and in fact to the early phase characterized by hyperplasia.

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Ethanol-Induced Artifacts in the Metabolism of ^{3}H-Vitamin D_3* (33962)

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There has been much recent activity in the area of the early biochemical events following the administration of vitamin D to D-deficient animals (1). In such experiments, of major concern is the route and vehicle of administration. Although the oral route represents a natural or physiologic one, it leaves much to be desired because absorption of the vitamin is a slow, irregular and incomplete process (1–4). Intraperitoneal and intramuscular routes are neither direct nor physiological. Intravenous administration certainly appears to offer the best route for such studies, but the vehicle used represents the major concern. Early studies in this laboratory employed Tween-20 suspensions; however artifacts in the case of the apparent liver metabolites soon became obvious. Ethanol has since been used with apparent success; but this unphysiological vehicle has now revealed some artifactual effects. Abnormalities in the plasma disappearance of tritiated vitamin D_3 injected in ethanol solvent will be demonstrated by comparison with the fate of the same dose injected in a plasma vehicle.

Materials and Methods. [1, 2-^{3}H]-vitamin D_3 (sp act 24,453 dpm/IU or 0.44 μCi/μg) synthesized in this laboratory according to a method previously reported (5), was used. Radiochemical purity was assessed by UV spectrophotometry and biological assay (rat line-test) (6). The radioactive vitamin D_3 for injection was dissolved in absolute ethanol or in plasma from vitamin D-deficient rats at a concentration of 200 IU/ml. The plasma was obtained after centrifugation of heparinized blood (25 USP units of heparin/ml) from vitamin D-deficient rats. The [1, 2-^{3}H]-D_3 in 0.05 ml of absolute ethanol was added to not less than 2 ml of plasma. In such a preparation, the important dilution of ethanol (1:40) in plasma avoids apparent precipitation of the plasma proteins.

The actual concentration of each iv solution was checked in each series of experiments by measuring the radioactivity of three aliquots equivalent to the dose injected (0.05 ml) and delivered in counting vials by the same syringe and needle as used for the

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