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EFFECT OF ISOPROTERENOL ON THE RAT SALIVARY GLAND FUNCTION

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Isoproterenol이 白鼠唾液腺 機能에 미치는 影響

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Abstract

Isoproterenol이 白鼠唾液腺의 機能에 미치는 影響을 觀察하기 爲하여 isoproterenol을 一回 或은 一日一回 五日間 皮下注射한 後 顎下腺과 耳下腺의 重量變動과 amylase活性度, nitrogen量 및 數種의 電解質變動을 관찰한 바 아래와 같은 結論을 얻었다.

1) Isoproterenol을 長期間 白鼠에 投與하면 唾液腺의 增大가 招來되는 바, 特히 顎下腺의 增大現象은 현저했으나 耳下腺은 거의 變動이 없었다.
2) 唾液腺內의 amylase 活性度는 isoproterenol 投與後 2時間에 가장 顯著히 增加되었으며 이후부터 점차 감소되어 24時間後에는 거의 正常으로 되었다.
그러나 isoproterenol 投與前 24時間부터 굶긴 群에서 耳下腺의 amylase 活性度는 isoproterenol 投與後 2時間에 顯著히 減少되었다.
3) 顎下腺內의 Ca 量은 isoproterenol 投與後 2時間에 가장 顯著히 減少되었고 이後 점차 增加되는 경향을 나타내었다. 特히 長期間 isoproterenol을 投與한 경우에는 마지막回 投與後 24時間경과군에서는 顎下腺內 Ca量이 오히려 증가 하였다.
4) 長期間 isoproterenol을 投與한 경우에 마지막회 投與後 24시간 經過群에서는 顎下線內 Mg와 Na量이 增加되었다.
5) 耳下腺內의 Mg量은 isoproterenol 投與로 增加되었다.

INTRODUCTION

Salivary gland enlargement, induced by chronic administration of isoproterenol, has deen reported in rats(1~5), mouse(6) and guinea pig(7). With regard to isoproterenol-inducedenlargement, Selye, Veilleux and Cantin have suggested that, at the rat, this is selective for salivary glands and results primarily from increased cellular proliferation. In other reports on the mouse, Brown-Grant(6) indicated that hypertrophy alone may account for the increased gland size.

A single injection of dl-isoproterenol causes, after a lag period of 20 hrs, a marked stimulation of DNA and RNA synthesis in the salivary glands of rodents (8—11). Also, MAO activity is increased by isoproterenol(12—14).

On the other hand, stimulation of mouse salivary gland by isoproterenol results in an initial decrease in peroxidase activity, followed by the subsequent return to normal values(15). Norepinephrine content of salivary gland is decreased by isoproterenol(16, 17).

The dual property of isoproterenol, that is, to produce both secretory and trophic effects, has been utilized in investigations of the kinetics of secretion and resynthesis of amylase in the normal rat parotid gland. The amylase in the rat salivary glands is secreted and synthesized as a result of isoproterenol injection(17—19).

A single injection of isoproterenol reduces submaxillary and parotid gland Ca level (20—22), but repeated subcutaneous administration of isoproterenol increases the Ca content of submaxillary gland to double the initial concentration (22, 23).

It is evident that Ca in the rat submaxillary gland is secreted to saliva by isoproterenol injection, because the losing Ca content from submaxillary gland by isoproterenol is identified with the increased Ca content of saliva by isoproterenol. (21, 22)

The rat submaxillary gland contains both alpha- and beta-receptors for catecholamines. Both types of receptors can be sensitized by previous secretion of the chorda-lingual nerve or by removal of the superior cervical ganglion. (24)

The experiments summarized in this report were designed to investigate the role of isoproterenol on the function of the rat salivary gland. Changes of amylase activity, nitrogen content and various electrolytes contents of rat salivary gland by isoproterenol were examined for elucidating the metabolic effects of isoproterenol on rat salivary gland.

METHODS

The rats used in this experiment were Korean Albino Females weighing 110—140gm. The rats were divided into two major groups. One group served as a control while the others received isoproterenol.

Isoproterenol treated groups were subdivided as the following; one minor group was sacrificed at 1hr, 2hrs., 3hrs, 12hrs and 24hrs after single injection of isoproterenol while the others after 5th injection of isoproterenol(single injection each day). Single injection dose of isoproterenol was 10mg/kg of body weight. Each group was consisted of 3 to 5 rats. Afetr the salivary glands were rapidly removed from the rats sacrificed by spinal dislocation and weighed, the left salivary glands were placed in dry oven to weigh the dry weight and the right salivary glands were minced. The dried left salivary glands were placed in the furnace of 500°C for 2hrs. These ashed salivary glands were used for determining the Na, Ca and Mg content. Ca content of salivary gland was assayed by Kovace method(27). Na and Mg content was assayed by direct flame photometry. The minced right salivary glands were homogenized in ice-cold saline, made up to 10ml and stored at 4°C until assay. Complete homogenization was found to be a critical step in obtaining reproducible results. 0.2ml of this homogenized solution was used for the determination of nitrogen content by Microkjeldahl method(25). The remainder of homogenates centrifuged and amylase activity was assayed immediately by Gomori

method. (26)

RESULTS

1) Effect of isoproterenol on the weight of salivary gland.

After single injection of isoproterenol, there was no changes in the weight of rat salivary gland. On the other hand, marked enlargement of rat salivary glands was resulted by chronic administration of isoproterenol, generally confirming the obsevations reported by Selye et al. (Table 1)

Table Ⅰ. Effect of isoproterenol on the rat salivary gland weight.

	No. of prep.	Body wt(gm)		Galnd Weight (mg)			
				Submaxillary		Parotid	
		Initical	Final	Wet wt.	Dry wt.	Wet wt.	Dry wt.
Control	5(3)*	118±3.4	129±4.8 (120±2.1)*	310.4±16.2 (319.7±49.7)*	37.2±2.2 (38.7±1.9)*	106.8±5.7 (97.3±9.9)*	11.8±0.4 (10.7±1.1)*
1hr after ISO single inj	3		126±1.5	308.0±19.8	30.0±1.6	110.3±4.0	10.5±0.3
2hrs after ISO single inj	3(3)*		125±2.1 (137±8.3)*	313.3±9.3 (264.7±18.4*)	30.0±0.4 (46.3±2.4)	107.0±3.7(1 14.0±4.9)*	10.8±0.4 (13.30±.2)
6hrs after ISO single inj	3		121±8.3	256.3±12.3	25.2±0.4	103.6±3.2	10.2±0.2
12hrs after ISO single inj	3		127±4.0	321.0±18.3	34.3±0.2	101.7±4.8	10.7±0.5
24hrs after ISO single inj	3		118±4.2	328.0±10.3	34.7±4.8	85.7±5.8	6.0±0.1
1hr after ISO 5th inj	3	114±8.4	119±1.0	472.0±8.7	48.5±2.4	94.0±4.9	10.0±0.1
2hrs after ISO 5th inj	3(3)*	114±5.7 (135±5.2)*	128±2.9 (129±9.1)*	469.3±38.7 (478.3±38.2)*	49.7±1.9 (36.7±2.0)*	106.6±5.2(1 20.7±11.3)*	11.0±1.4 (12.3±0.3
6hrs after ISO 5th inj	3	122±4.4	122±3.3	623·0±47.9	74.3±3.7	104.7±7.8	11.7±1.0
12hrs after ISO 5th inj	3	112±3.9	110±4.8	417.0±38.3	56.0±1.8	89.7±2.7	11.7±0.4
24hrs after ISO 5th inj	3	116±3·7	117±1.9	647.0±48.3	67.7±8.8	105.5±5.5	13.0±0.7

ISO means isoproterenol.
( )* : 24hrs starvation group before isoproterenol injection.
G. W. : Gland Weight

The submaxillary glands exhibited approximately 2-fold increase but the parotid glands no changes compared with non-treated groups.

2) Effect of isoproterenol on the amylase activity of salivary gland.

Amylase activity of salivary gland was increased maximally at 2hrs after isoproterenol injection, and then decreased approximately to the normal activity at 24hrs after isoproterenol injection. In animals starved for 24hrs before isoproterenol injection, amylase activity was reduced severly at 2hrs after single or 5th injection of isporoterenol(Table Ⅱ).

3) Effect of isoproterenol on nitrogen content of salivary gland.

Nitrogen content was decreased slightly in submaxillary gland at 1 hrs after single or 5th injection of isoproterenol but not in parotid gland. In animals starved for 24hrs before isoproterenol injection, nitrogen concentration of salivary glands was reduced upto approximately half of initial concentration at 2hrs after injection of isoproterenol(Table Ⅲ).

Table Ⅱ. Effect of isoproterenol on the amylase activity of rat salivary gland.

	No. of prep.	Amylase activity (units/100Gm of G.W.)	
		Submaxillary	parotid
Control	5(3)*	993±262.0 (3446±288.2)*	1560±321.2 (11057±108.4)*
1hr after ISO single inj	3	2988±500.2	1600±249.3
2hrs after ISO single inj	3(3)*	9383±336.7 (3942±530.3)*	12868±4130.3 (2412±330.9)*
6hrs after ISO single inj	3	1240±330.9	7999±5320.2
12hrs after ISO single inj	3	1989±327.7	1713±339.2
24hrs after ISO single inj	3	1212±142.5	3058±439.6
1hrs after ISO 5th inj	3	9690±198.2	12814±320.8
2hrs after ISO 5th inj	3(3)*	27844±407.5 (2899±479.2)*	18697±579.8 (4683±432.2)*
6hrs after ISO 5th inj	3	14188±301.1	9798±278.3
12hrs after ISO 5th inj	3	3155±178.1	6533±270.8
24hrs after ISO 5th inj	3	4267±108.4	6493±109.1

ISO means isoproterenol.
( )* : 24hrs starvation group before ISO injection.
G. W. : Gland Weight

Table Ⅲ. Effect of isoproterenol on the N content of rat salivary gland.

	No. of prep.	Nitrogen (mg/100Gm of G.W.)	
		Submaxillary	Parotid
Control	5(3)*	42.62±2.4 (41.9±0.8)*	49.8 ±2.8 (48.9±1.2)*
1hrs after ISO single inj	3	33.9±1.4	43.2±0.3
2hrs after ISO single inj	3(3)*	39.0±1.7 (35.4±0.5)*	42.4±0.7 (29.5±1.8)*
6hrs after ISO single inj	3	41.1±1.3	47.0±1.0
12hrs after ISO single inj	3	41.4±0.7	53.0±2.3
24hrs after ISO single inj	3	42.3±1.2	43.5±2.8
1hrs after ISO 5th inj	3	38.7±0.3	46.5±1.3
2hrs after ISO 5th inj	3(3)*	34.9±4.2 (19.8±7.9)*	41.9±5.7 (25.5±1.2)*
6hrs after ISO 5th inj	3	42.0±1.1	42.6±1.2
12hrs after ISO 5th inj	3	41.9±1.1	53.6±1.7
24hrs after ISO 5th inj	3	51.4±0.6	43.1±2.4

ISO means isoproterenol
( )* : 24hrs starvation group before ISO injection.
G.W. : Gland Weight

4) Effect of isoproterenol on the electrolyte contents of salivary glands.

Single injection of isoproterenol caused a marked loss of Ca from the submaxillary gland. The loss of Ca was maximal at 2hrs and reaccumlation of Ca began since this time. The Ca

content of submaxillary gland was almost same as the normal at 24 hrs after isoproterenol injection(Tabie Ⅳ).

Table Ⅳ. Effect of isoproterenol on the Ca content of rat salivary gland.

	No. of prep.	Ca (mEq/KG of G.W.)	
		Submaxillary	Parotid
Control	5(3)*	13.9±1.2 (12.7±2.8)*	11.4±3.7 (12.0±1.2)*
1hr after ISO single inj.	3	2.7±0.37	12.7±3.3
2hrs after ISO single inj.	3(3)*	2.7±0.7 (6.9±1.3)*	14.7±2.1 (11.9 ±1.2)*
6hrs after ISO single inj.	3	5.7±1.2	12.5±1.4
12hrs after ISO single inj.	3	5.7±1.0	9.8±0.7
24hrs after ISO single inj.	3	14.9±1.0	11.7±1.0
1hrs after ISO 5th inj.	3	7.9±0.2	14.8±0.7
2hrs after ISO 5th inj.	3(3)*	4.7±0.3 (3.8±0.7)*	17.7±1.4 (7.5±1.3)*
6hrs after ISO 5th inj.	3	5.6±1.9	12.3±3.1
12hrs after ISO 5th inj.	3	22.1±1.7	10.9±1.2
24hrs after ISO 5th inj.	3	44.8±0.6	1.0±0.2

ISO means isoproterenol

( )* : 24hrs starvation group before ISO injection.

G.W. : Gland Weight.

Table Ⅴ. Effect of isoproterenol on the Mg and Na content of rat salivary gland.

	No. of prep.	Mg (mEq/KG of G.W.)		Na(mEq/KG of G.W.)
		Submaxillary	Parotid	Submaxillary
Control	5	22.0±1.3	36.0±3.0	34.8±2.5
1hr after ISO single inj.	3	22.7±1.0	48.0±5.7	76.0±6.4
2hrs after ISO single inj.	3	23.7±2.0	32.0±1.8	78.3±2.7
6hrs after ISO single inj.	3	20.1±1.2	24.0±3.3	73.0±7.9
12hrs after ISO single inj.	3	21.0±0.3	39.0±2.7	73.7±1.7
24hrs after ISO single inj.	3	27.0±3.6	53.3±7.2	61.7±4.8
1hr after ISO single inj.	3	20.5±0.3	48.8±0.8	42.0±1.7
2hrs after ISO 5th inj.	3	16.0±1.8	45.3±4.7	35.0±4.9
6hrs after ISO 5th inj.	3	30.9±2.2	57.0±4.4	44.3±5.7
12hrs after ISO 5th inj.	3	25.0±0	47.3±3.3	71.0±3.2
24hrs after ISO 5th inj.	3	33.5±2.0	47.5±2.0	72.0±1.0

ISO means isoproterenol

G.W. : Gland Weight

The chronic administration of isoproterenol produced a marked increase in Ca contents of submaxillary glands at 24hrs after the last injection.

Na contents of submaxillary glands increased strikingly after acute stimulation but increased slightly at 12 and 24 hrs after the last injection of isoproterenol in the case of chronic admin-istration.

Mg contents of salivary glands were not changed by the administration of isoproterenol.

DISCUSSION

The results obtained confirmed the observation that the chronic administration of isoproterenol casuses enlargement of salivary glands of rats(1—5) and elucidated the nature and organ specificity of action.

Selye, vellieux and Cantin(1) reproted a marked increase in mitotic figures as well as in cell size of enlarged salivary glands, but considered the enlargement to be primarily the result of cellular prolileration. Brown-Grant (6), however, found no clear evidence of increased mitosis in isoproterenol-enlarged salivary glands of mouse. Schneyer(2) showed the presence of mitotic figures early in the period of isoproterenol treatment but failed to support the occurence of appreciable cell proliferation. And an increase in cell size sufficient to account for the increase in gland size was observed.

Byrt(18) reported the concentration of amylase in parotid gland was decreased to about a sixth of its original concentration 2hrs after isoproterenol injection and then increased to the normal level. On the other hand, the amylase activity of salivary gland was increased to about 10 times of its original activity 2hrs after single isoproterenol injection in this experiment and in the case of chronic administration increased to about 12—30 times of its original activity 2hrs after the last injection. This phenomena can be suggested to result in the fact that the salivary amylase was secreted by night feeding, because the animals were not starved for 24hr before the injection of isoproterenol.

Because isoproterenol has dual properties, that is, to secret and synthetize amylase(17—19), amylase activity was increased after isoproterenol injection in the case of not starving for 24hrs before isoproterenol injection, but it was decreased to about a half and quarter of its original activity respectively at 2hrs after single and 5th injection of isoproterenol in groups starved for 24hrs prior to the injection of isoproterenol. At 24hrs after chronic administration of isoproterenol, the amylase activity of salivary glands was increased to approximatly 5 times of its original activity. This fact is buttered by the report that a single injection of dl-isoproterenol causes, after a lag period of 20hrs, a marked stimulation of DNA and RNA synthesis in the salivary gland of rodents(8—11).

Isoproterenol injection caused even more striking depletion of Ca in submaxillary glands of rats than pilocarpine injection (21). The greater depletion of Ca by isoproterenol probably results from a great loss of secretory granules after administration of sympathomimetic agents than after parasympathomimetic agents(28).

The rapid loss of Ca from the submaxillary gland indicates that Ca was held in a rapidly mobilziable form. The secretion of Ca in saliva after isoproterenol was chiefly remarkable for the great ratio between saliva Ca and the ultrafiltrable Ca of serum.

If Ca in the isoproterenol-stimulated saliva is present in ionic form, a barrier to free diffusion

of Ca out of the duct system must be present. Another possibility which might account for the great decrease of Ca in submaxillary gland in this experiment is the presence of a Ca binding substance in the gland which prevents diffusion. Such a substance could be liberated from secretory granules at the same time Ca is lost from the gland.

In the case of single injection of isoproterenol, not only Ca content but also Mg, sialic acid, protein and N content were decreased(29). This report is similar to this data in Ca, Mg and N content changes. Repeated administration of isoproterenol induced a rapid increase in both weight and Ca content of submaxillary glands and in weight of parotid gland but no change in other glands(23). In this experiment, Ca, nitrogen, Mg and Na contents of submaxillary gland was increased to about 4, 1.5, 1.6, and 2 times of its original contens respectively 24 hrs after chronic isoproterenol administration.

SUMMARY

To investigate the role of isoproterenol on the function of rat salivary gland, the authors examined the changes of amylase activity, nitrogen content and several electrolytes contents in the salivary gland of rats after isoproterenol injection.

The results were as follow:

1) Enlargement of salivary glands was observed. Salivary glands were differently affected, with submaxillary showing the more, and parotid the least after chronic isoproterenol administration.

2) Amylase activity of salivary glands was increased maximally at 2hrs after isoproterenol injection, and then decreased almost to the normal activity at 24hrs after injection. In animals starved 24hrs before isoproterenol injection, amylase activity of parotid gland was markedly reduced at 2 hrs after single and 5th injection of isoproterenol.

3) Ca content of submaxillary gland was decreased maximally at 2 hrs after isoproterenol injection, and then returned to the normal value at 24 hrs after the last injection of isoproterenol in the case of chronic administration.

4) Mg and Na contents of submaxillary glands was increased at 24 hrs after the last injection in chronic administration.

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