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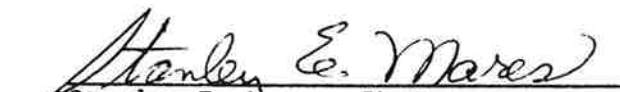
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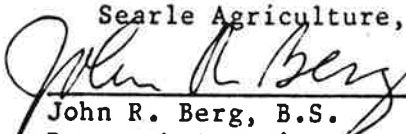
Effects of Aspartame (SC-18862)
on Gonadotropin Secretion in Rats

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Introduction

A number of peptides such as gonadotropin releasing hormone and its analogs have been shown to exert profound effects on the secretion of gonadotropins by the pituitary gland (1,2,3). Although SC-18862 bears no structural resemblance to these peptides, it was of interest to determine any possible effects of this peptide on gonadotropin secretion. This report describes two experiments in which SC-18862 was evaluated for its effects on pituitary secretion of LH and FSH, as well as prolactin, in rats. It has been reported (4) that the probable maximum daily ingestion of aspartame would be about 1.3 to 1.7 grams. This corresponds to the consumption of approximately 22 to 28 mg/kg/day for a 60 kg person. The doses of SC-18862 used in the present study ranged up to ten times the above estimated adult human daily intake.

Methods

Experiment 1

The animals used were obtained from Charles River Breeding

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Laboratories, Inc., of Wilmington, Mass. Both male and female animals were of the Crl: COB® CD® (SD) strain of albino rats. In this experiment 30 female and 30 male rats were randomly segregated into groups of 10 animals each. The female rats were ovariectomized one day prior to treatment. A group of male rats and a group of female rats were used for each treatment. Treatments consisted of vehicle only (controls), 100 mg/kg/day of SC-18862 and 300 mg/kg/day of SC-18862 administered once daily intragastrically using a suspension in corn oil. The rats were weighed initially and again on day 6 of treatment and the dosages were adjusted for any changes in body weight. The duration of treatment was 10 days. Blood samples (2 ml) were obtained under ether anesthesia from a jugular vein on the mornings of day 6 and day 10. All rats were weighed and sacrificed on day 10 after blood samples were taken.

Experiment 2

In this experiment 30 male rats were used. They were randomly divided into 3 groups of 10 rats each. SC-18862 was administered intragastrically suspended in corn oil and the dose regimens were identical to those in the first experiment. The duration of treatment was 10 days and blood samples (2 ml) were taken under ether anesthesia on day 11, one day after the last treatment. All the rats were sacrificed on day 11 after blood sampling.

In both experiments blood samples were allowed to clot at room temperature and serum samples were obtained after centrifugation at 1000 x g for 30 minutes at 4°C. The serum samples were stored at -20°C until analyzed by radioimmunoassay for LH, FSH and prolactin in the first experiment and FSH

only in the second experiment.

On the day of autopsy the animals were sacrificed using carbon dioxide asphyxiation. Pituitary glands were rapidly removed, weighed and homogenized in 4 ml of 1% ovalbumin in 0.1M phosphate-buffered saline, pH 7.4, followed by two 4-ml rinses with the same buffer. These homogenates were frozen immediately on dry ice and stored at -20°C until evaluation by radioimmunoassay. The wet weights of the uteri, seminal vesicles, ventral prostates and pituitary glands were obtained. In addition, testes weights were obtained in the second experiment.

Radioimmunoassays for LH, FSH and prolactin were performed using the homologous double-antibody method described by Niswender et al. (4) with materials supplied by the National Institute of Arthritis, Metabolic and Digestive Diseases (NIAMDD). Blood serum and pituitary samples were each analyzed using two different size aliquots. The data were analyzed using a logit-log transformation and the samples were also examined for parallelism of response in comparison to standards. The results are reported in terms of nanogram equivalents of rat LH-RP-1 (0.03 x NIH-LH-S1) and rat FSH-RP-1 (2.1 x NIH-FSG-S1) and rat prolactin-RP-1 (11 IU/mg). Differences between groups were tested for statistical significance using a Student's t test at the (two-tailed) $P \leq 0.05$ level.

Results

Experiment 1

Intragastric administration of SC-18862 at daily doses of 100 or 300 mg/kg for ten days had no significant effects on body weight gains or weights

of the pituitary gland or uterus in ovariectomized female rats (Table 1). Likewise the same treatments had no significant effects on body weight or weights of the pituitary gland, seminal vesicles or ventral prostate glands in male rats (Table 2). Blood serum levels of LH, FSH or prolactin in the ovariectomized female rats were unaffected on either day 6 or day 10 of the treatment (Table 3). Pituitary gland levels of LH, FSH and prolactin were also unaffected at the end of the ten days of treatment. In male rats there were no significant effects on blood serum levels of FSH or prolactin with the exception of an increase in FSH on day 10 after the 100 mg/kg/day dosage (Table 4). However, this increase was not considered biologically important because FSH levels were unaffected at the higher dosage of 300 mg/kg/day. Pituitary gland levels of LH, FSH and prolactin were unaltered.

In agreement with other studies (5,6), measurement of serum LH in intact male rats was difficult because baseline levels were often undetectable. Measurable levels of LH were found in only 6 of 60 serum samples analyzed in these groups. For this reason, quantitative statistical evaluation was not applied to these values.

Experiment 2

In this experiment male rats were given the same treatment as in experiment 1, but the body and organ weights were determined one day after the last day of treatment. There were no significant effects on body weight or weights of the pituitary gland, testes or seminal vesicles. There was a slight, though statistically significant, reduction in the weight of the

Table 1.

Effects of SC-18862 on Body Weight and
Organ Weights in Ovariectomized Female Rats (Experiment 1)

Treatment	Dose i.g. mg/kg/day x 10 days	No. of Rats	Body Weight		Pituitary	Uterus
			Initial	Final		
			g	g	mg	mg
			mean \pm S.E.	mean \pm S.E.	mean \pm S.E.	mean \pm S.E.
Controls	--	10	219 \pm 3.1	270 \pm 3.4	12.5 \pm 0.33	119 \pm 6.8
SC-18862	100	10	216 \pm 5.4	267 \pm 8.5	13.1 \pm 0.46	112 \pm 6.6
	300	10	222 \pm 4.2	268 \pm 5.9	12.8 \pm 0.25	125 \pm 6.3

Note: Means of treated groups did not differ significantly from means of controls as determined by Student's t test ($P > 0.05$).

Searle data reference: RB 1418/235,236

Table 2.

Effects of SC-18862 on Body Weight and
Organ Weights in Male Rats (Experiment 1)

Treatment	Dose i.g. mg/kg/day x 10 days	No. of Rats	Body Weight		Pituitary	Seminal Vesicles		Ventral Prostate	
			Initial g	Final g		mg mean \pm S.E.	mg mean \pm S.E.	mg mean \pm S.E.	mg mean \pm S.E.
Controls	--	10	383 \pm 4.4	410 \pm 4.5	12.4 \pm 0.47	304 \pm 19.5	405 \pm 17.5		
SC-18862	100	10	391 \pm 10.6	419 \pm 11.5	13.2 \pm 0.47	311 \pm 11.2	372 \pm 31.7		
	300	10	386 \pm 5.6	422 \pm 7.1	12.4 \pm 0.26	292 \pm 13.6	439 \pm 20.1		

Note: Means of treated groups did not differ significantly from means of controls as determined by Student's
t test ($P > 0.05$).

Searle data reference: RB 1418/235,237

Table 3.

Effects of SC-18862 on Blood Serum and
Pituitary Gonadotropin Levels in
Ovariectomized Female Rats (Experiment 1)

Treatment mg/kg/day x 10 days, i.g.	Blood Serum		Pituitary μg/gland mean ± S.E.
	Day 6	Day 10	
	ng/ml mean ± S.E.	ng/ml mean ± S.E.	
LH			
Controls	287 ± 46 (10) ⁽¹⁾	379 ± 56 (10)	663 ± 53 (10)
SC-18862 100	245 ± 34 (10)	365 ± 35 (10)	621 ± 68 (10)
SC-18862 300	176 ± 34 (10)	315 ± 51 (10)	570 ± 58 (10)
FSH			
Controls	1420 ± 54 (10)	1409 ± 54 (10)	137 ± 7 (10)
SC-18862 100	1352 ± 87 (10)	1435 ± 89 (10)	157 ± 7 (10)
SC-18862 300	1395 ± 58 (10)	1443 ± 114 (10)	141 ± 7 (10)

(Table 3 continued)

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(Table 3 continued)

Treatment mg/kg/day x 10 days, i.g.	Blood Serum		Pituitary μ g/gland mean \pm S.E.
	<u>Day 6</u> ng/ml mean \pm S.E.	<u>Day 10</u> ng/ml mean \pm S.E.	
PROLACTIN			
Controls	30 \pm 3.8 (10)	45 \pm 9.7 (10)	39 \pm 5.5 (10)
SC-18862 100	27 \pm 8.5 (10)	33 \pm 5.3 (10)	40 \pm 4.9 (10)
SC-18862 300	27 \pm 3.4 (10)	45 \pm 14.0 (10)	37 \pm 5.5 (10)

(1) Values in parenthesis indicate the number of serum samples or pituitary glands analyzed.

Note: Means of treated groups did not differ significantly from means of concurrent controls as determined by Student's t test ($P > 0.05$).

Searle data reference: RB 1626/91-94,96,97

Table 4.

Effects of SC-18862 on Blood Serum and
Pituitary Gonadotropin Levels in Male Rats (Experiment 1)

Treatment mg/kg/day x 10 days, i.g.	Blood Serum		Pituitary μg/gland mean ± S.E.
	Day 6 ng/ml mean ± S.E.	Day 10 ng/ml mean ± S.E.	
LH			
Controls	ND ⁽¹⁾	ND	346 ± 28 (10) ⁽²⁾
SC-18862 100	ND	ND	286 ± 18 (9)
SC-18862 300	ND	ND	254 ± 42 (10)
FSH			
Controls	337 ± 56 (8)	302 ± 53 (10)	183 ± 8 (10)
SC-18862 100	444 ± 56 (10)	454 ± 44 ⁽³⁾ (10)	192 ± 7 (10)
SC-18862 300	315 ± 35 (9)	401 ± 38 (10)	196 ± 11 (10)

(Table 4 continued)

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(Table 4 continued)

Treatment mg/kg/day x 10 days, i.g.	Blood Serum		Pituitary
	<u>Day 6</u>	<u>Day 10</u>	
	ng/ml mean ± S.E.	ng/ml mean ± S.E.	µg/gland mean ± S.E.
PROLACTIN			
Controls	78 ± 11 (10)	108 ± 24 (10)	18 ± 1.4 (10)
SC-18862 100	78 ± 11 (10)	130 ± 22 (10)	17 ± 1.7 (10)
SC-18862 300	75 ± 8 (10)	120 ± 12 (10)	16 ± 1.4 (10)

- (1) ND = Not Detectable, i.e., most samples were below detectable assay levels (<50 ng/ml). Only 1 of 10 control rats (60 ng/ml) treated with 100 mg/kg/day showed detectable levels on the 6th day. None of the rats treated with either 100 or 300 mg/kg/day showed detectable levels on the 6th day. Only 4 of 10 control rats (72, 103, 112 and 290 ng/ml) and 1 of 10 rats (96 ng/ml) treated with 100 mg/kg/day showed detectable levels on the 10th day. None of the rats treated with 300 mg/kg/day showed detectable levels on the 10th day.
- (2) Values in parentheses indicate the number of blood serum samples or pituitary glands analyzed.
- (3) Significantly greater than control mean by Student's t test ($P < 0.05$).
Searle data reference: 1418/235.

ventral prostate following treatment with the high dose (300 mg/kg/day) of SC-18862 (Table 5). This effect, however, was not observed after the 100 mg/kg/day dosage. Blood serum and pituitary gland levels of FSH were unaffected by 10 days of treatment with 100 or 300 mg/kg/day (Table 6).

Conclusions

It is concluded from these experiments in ovariectomized female rats and in intact male rats that daily administration of SC-18862 at doses of 100 or 300 mg/kg for 10 days has no marked effects on secretion of gonadotropins by the pituitary.

Table 5.

Effects of SC-18862 on Body Weight and
Organ Weights in Male Rats (Experiment 2)

Treatment	Dose i.g. mg/kg/day x 10 days	No. of Rats	Body Weight		Pituitary	Testes		Seminal Vesicles		Ventral Prostate	
			Initial	Final		g	mean \pm S.E.	mg	mean \pm S.E.	mg	mean \pm S.E.
			g	g							
			mean \pm S.E.	mean \pm S.E.							
Controls	--	10	259 \pm 2.0	332 \pm 5.1	12.4 \pm 0.34	3.1 \pm 0.04		277 \pm 12.0		353 \pm 18.0	
SC-18862	100	10	263 \pm 1.9	335 \pm 3.9	12.5 \pm 0.57	3.1 \pm 0.11		286 \pm 10.5		350 \pm 11.3	
	300	10	264 \pm 3.5	337 \pm 8.1	12.8 \pm 0.29	3.1 \pm 0.07		265 \pm 9.8		306 \pm 13.6 ⁽¹⁾	

(1) Significantly different from control mean by Student's t test ($P \leq 0.05$).

Searle data reference: RB 1607/196-198

Table 6.

Effects of SC-18862 on Blood Serum and
Pituitary FSH Levels in Male Rats One
Day after Ten Days of Treatment (Experiment 2)

Treatment mg/kg/day x 10 days, i.g.	No. of Rats	<u>Blood Serum FSH</u> ng/ml mean \pm S.E.	<u>Pituitary FSH</u> μ g/gland mean \pm S.E.
Controls	10	614 \pm 47	150 \pm 14
SC-18862 100	10	543 \pm 26	152 \pm 7
SC-18862 300	10	568 \pm 38	148 \pm 7

Note: Treated group means did not differ significantly from control means as determined by Student's t test ($P > 0.05$).

Searle data reference: RB 1607/196; 1626/112

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