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Reduced Disease in Aged Rats Treated Chronically With Ibopamine, a Catecholaminergic Drug

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WALKER, R. F., C. A. WEIDEMAN AND E. B. WHEELDON. *Reduced disease in aged rats treated chronically with ibopamine, a catecholaminergic drug*. NEUROBIOL AGING 9(3) 291–301, 1988.—As part of preclinical safety testing for carcinogenicity, postpubertal (50 days old) rats were dosed (0, 30, 90 or 180 mg/kg/day) with ibopamine (N-methyldopamine, 0,0'-diisobutyroyl ester·HCl; SK&F 100168) for 730 consecutive days. Neoplastic and nonneoplastic lesions were identified histologically in all rats that died during the period of dosing, as well as in those that were killed after it was completed. Six neoplastic lesions (adrenal cortical, mammary, and pituitary adenoma, skin papilloma, pheochromocytoma and mammary adenocarcinoma) and five nonneoplastic lesions (chronic glomerulonephropathy, renal pelvic mineralization, hepatocellular proliferative nodule, galactoceles and chronic cardiomyopathy) were significantly reduced in a dose-related fashion in at least one sex of ibopamine-treated rats. In addition, age-related alopecia and atrophy of the adrenal zona glomerulosa were retarded by ibopamine treatment. Squamous cell skin carcinoma was the only lesion that was significantly ($p<0.05$) increased in the treated groups. Mortality during the study was not significantly different in treated and control groups, indicating that the lower incidence of disease in ibopamine-treated rats was a drug effect and not an artifact of differential survival. Although life span was not measured, ibopamine-treated rats had significantly less malignant lesions than controls at the end of dosing, suggesting a potentially positive effect of treatment on population survival. As the result of these beneficial effects, ibopamine may be useful for future study of factors affecting the occurrence of disease during aging.

Ibopamine Catecholamines Antitumor Antiaging Reduced disease

CATECHOLAMINES are progressively lost from the central nervous system of rodents [12], monkeys [13] and humans [32] during aging. This change in monoamine composition of the mammalian brain may contribute to physical and psychological deterioration since pharmacologic replacement of catecholamines improved movement [21], cognitive behavior [2] and extended fertility [9] in old animals. Chronic dietary administration of the catecholamine precursor levodopa (1-3,4-dihydroxyphenylalanine) also extended longevity in mice, presumably by "reducing the incidence of intercurrent disease" during aging [9].

A causal relationship between disease, catecholamine decrements, and premature death in humans was suggested by the fact that patients with Parkinson's disease, a nonlethal affliction resulting from loss of nigrostriatal dopamine neurons, had higher mortality than age-matched subjects from the general population [14]. Life expectancy in the Parkinson's patients increased to normal values upon treatment with levodopa which replaced catecholamines that were lost as a result of the disease [19,30]. Life span also increased significantly in mice that were chronically treated with levodopa, suggesting that catecholamine decrements contribute to the onset and progression of disease during aging [9].

As part of preclinical safety testing for carcinogenicity, ibopamine (SK&F 100168-A), the diisobutyroyl ester of N-methyl-dopamine, was administered daily to rats for two years. The compound is orally active, and exerts its pharmacologic effects through differential, dose-dependent activation of dopaminergic, alpha-adrenergic and/or beta-adrenergic receptors. For example, in rats the diuretic effect of ibopamine is antagonized by dopaminergic and alpha adrenergic receptor antagonists but not by beta receptor antagonists, while the increased blood pressure effect is antagonized by alpha but not beta or dopaminergic receptor blockers [11].

The duration of drug administration in the present study constituted the major portion of a rat's life span, providing an opportunity to test the effects of ibopamine on the spontaneous occurrence of diseases whose incidences increase with age. Since ibopamine reduced the frequency of several neoplastic and nonneoplastic lesions, it may represent a class of catecholaminergic drugs with the potential to alleviate some of the intrinsic diseases of senescence.

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APPENDIX I: SAMPLE SIZES

Organ	Group	Males Ibopamine (mg/kg/day) 0	30	90	180	Females Ibopamine (mg/kg/day) 0	30	90	180
Adrenal Gland	Found Dead	98	46	47	55	111	59	60	51
	Killed Terminally	102	53	52	45	89	41	40	49
Brain	Found Dead	98	—	—	55	111	—	—	51
	Killed Terminally	102	—	—	45	89	—	—	49
Duodenum	Found Dead	—	—	—	—	111	—	—	51
	Killed Terminally	—	—	—	—	89	—	—	49
Heart	Found Dead	98	48	48	55	111	59	60	51
	Killed Terminally	102	8	4	45	89	0	3	49
Jejunum	Found Dead	98	—	—	54	—	—	—	—
	Killed Terminally	100	—	—	45	—	—	—	—
Kidney	Found Dead	98	46	48	55	111	59	60	51
	Killed Terminally	102	20	20	45	89	11	11	49
Liver	Found Dead	98	46	48	55	111	59	60	51
	Killed Terminally	102	33	30	45	89	18	20	49
Lung	Found Dead	98	—	—	55	—	—	—	—
	Killed Terminally	102	—	—	45	—	—	—	—
Mammary Gland	Found Dead	98	46	48	55	111	59	60	51
	Killed Terminally	102	3	3	45	89	27	19	49
Pancreas	Found Dead	98	—	—	54	—	—	—	—
	Killed Terminally	102	—	—	45	—	—	—	—
Parathyroid Gland	Found Dead	94	—	—	55	94	—	—	40
	Killed Terminally	87	—	—	37	68	—	—	39
Pituitary Gland	Found Dead	95	46	48	55	111	59	60	51
	Killed Terminally	102	27	23	45	89	29	24	49
Skin	Found Dead	98	46	47	55	110	59	60	51
	Killed Terminally	101	48	47	44	89	31	22	49
Stomach	Found Dead	98	—	—	55	—	—	—	—
	Killed Terminally	102	—	—	45	—	—	—	—
Testes	Found Dead	98	46	48	55				
	Killed Terminally	102	9	8	45				
Thyroid Gland	Found Dead	98	—	—	55	111	—	—	51
	Killed Terminally	102	—	—	45	89	—	—	49
Uterus	Found Dead					111	—	—	51
	Killed Terminally					89	—	—	49

METHOD

Outbred rats [CD(BR)SD; Charles River Labs, Wilmington, DE] were used in this study because historical data provided extensive information on lesion incidences in this strain. The animals were housed individually in wire cages and were acclimated to standard laboratory conditions of light (12 hours; 0600–1800 hr), temperature (75±5°C) and nutrition (Wayne Certified Lab-Blox-Meal and water ad lib for two weeks after which 500 males and 500 females were randomly assigned to the study [8]. Ibopamine was administered daily by gavage as an aqueous solution containing 0, 30, 90 and 180 mg/kg/day. Each dosing group contained 100 rats (50 male, 50 female) except for controls (0 mg/kg/day) which consisted of two groups containing 100 rats each. Two control groups were used to compensate for anticipated variability in the spontaneous occurrence of neoplasms in long-term studies [25]. The additional control group provided data to calculate a normal range of lesion frequencies against which to assess the incidence of pathological changes and their significance in the drug-treated rats.

Ibopamine treatment lasted for two years (730 days) up to but not including the day of necropsy. All animals received daily health checks, and records of their body weights, food consumption and general physical health, as well as pharmacologic and toxicologic effects of ibopamine, were maintained. Physical examinations were performed monthly with

TABLE 1

BODY WEIGHT AND FOOD CONSUMPTION IN IBOPAMINE-TREATED RATS PRESENTED AS % OF CONTROL AVERAGE

Duration of Treatment	Males Ibopamine (mg/kg/day)			Females Ibopamine (mg/kg/day)		
	30	90	180	30	90	180
Body Weight						
6 months	100.2	94.9	94.7	98.2	100.3	97.0
12 months	100.6	93.9	90.0	99.0	96.8	91.2
18 months	99.7	91.2	87.4	95.4	95.0	88.2
24 months	104.0	97.7	93.7	99.4	96.1	91.4
Food Consumption						
6 months	105.4	98.4	96.2	97.9	94.4	90.8
12 months	104.2	107.7	104.2	103.9	109.4	100.8
18 months	104.7	95.9	95.3	90.2	87.1	94.7
24 months	103.3	100.5	103.3	96.7	94.1	100.7

Body weights of male and female rats (180 mg/kg/day) were significantly lower ($p<0.05$, at least) than controls after 6 to 24 months of ibopamine treatment.

particular attention given to the occurrence of palpable tissue masses. Moribund animals were immediately euthanized and necropsied. After two years of dosing all remaining animals were killed and all major organs were collected. All tissues from animals in control and high-dose groups, as well as those from all rats that died during the study, were examined microscopically. The incidences of lesions in these tissues were then compared statistically using Fisher's Exact test [18]. The statistical significance of dose response trends in the incidences of lesions when all dose groups were examined was assessed with the Cochran Armitage trend test [1,7]. Body weight and food consumption data were analyzed using the Student's *t*-statistic [28]. The specific numbers of animals from which the incidence data were calculated for lesions occurring in those found dead or killed terminally are presented in Appendix I.

RESULTS

The incidences of various lesions in the two control groups were not statistically different. Thus, the data from both groups were combined for comparing and assessing the effects of ibopamine on the occurrence of neoplastic and nonneoplastic lesions in test animals.

The data presented in Fig. 1 show the cumulative mortality of male and female rats assigned to vehicle and ibopamine treatment groups. Temporal differences in survival between the various groups were not statistically significant.

The body weights and amounts of food consumed by rats used in the present study are summarized in Table 1. Body weights were not significantly different between ibopamine-treated and control rats of either sex at the onset of dosing. However, after receiving ibopamine (180 mg/kg/day) for 6 months, male rats weighed significantly less ($p<0.05$) than their concurrent controls. After 6 months of treatment, female rats also weighed significantly less ($p<0.05$) than their controls. The reductions in body weight did not remain constant or increase uniformly, but instead, fluctuated between 1% and approximately 12% until the study was terminated. The differences in body weight between ibopamine-

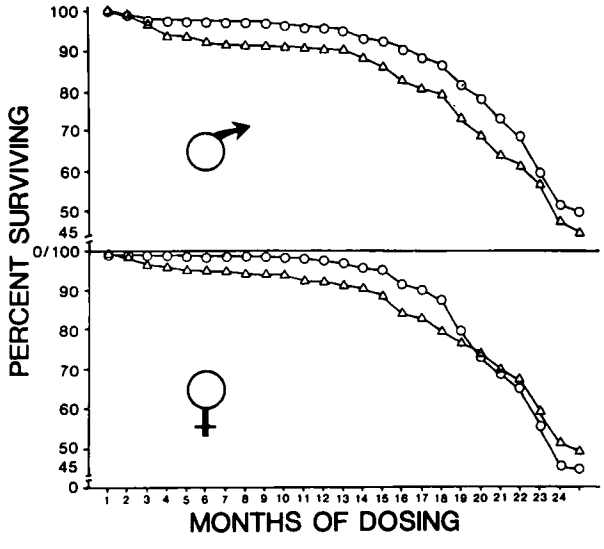

FIG. 1. Survival curves for rats treated with ibopamine (△; 180 mg/kg/day) or vehicle (○).

treated and control rats did not correlate with food consumption. For example, after 12 months of ibopamine treatment at 180 mg/kg/day, male rats weighed 10% less but ate 4% more than their controls. After 24 months of dosing, the same group of rats weighed 6% less than controls even though they consumed 3% more food. Similar differences were observed in other dose groups. However, differences in food consumption between ibopamine-treated and control groups were not statistically significant.

As seen in Table 2, the incidences of neoplasms, whether benign, malignant or multiple, in ibopamine-treated rats of both sexes were significantly lower ($p<0.01$, at least) than controls. The data in Table 2 were compiled from counts of all lesions that were observed during histological examination of all tissues, whether or not the incidence of each spe-

TABLE 2

EFFECT OF IBOPAMINE ON THE INCIDENCE OF NEOPLASMS IN RATS

	Incidence (% of Animals)			
	Male		Female	
	Control	Ibopamine*	Control	Ibopamine*
Number of Animals	200	100	200	100
Neoplasms	88%	76%†	97%	86%‡
Benign Neoplasms	80%	63%†	96%	83%‡
Malignant Neoplasms	53%	33%‡	39%	25%†
Multiple Neoplasms	62%	34%‡	75%	48%‡

*Ibopamine was administered in doses of 180 mg/kg/day.
†Comparison with control group is statistically significant ($p<0.01$).
‡Comparison with control group is statistically significant ($p<0.001$).

TABLE 3

EFFECT OF IBOPAMINE ON THE OCCURRENCE OF BENIGN LESIONS IN MALE RATS

		Decreased Incidence Ibopamine (mg/kg/day)		Equal or Increased Incidence Ibopamine (mg/kg/day)	
Lesion	Group	0	180	0	180
Adrenal Cortical Adenoma	Found Dead	23.5%	5.5%†		
	Killed Terminally	26.5%	15.6%		
	Total	25.0%	10.0%†		
Mammary Gland Adenoma	Found Dead	1.0%	0.0%		
	Killed Terminally			0.0%	0.0%
	Total	0.5%	0.0%		
Mammary Gland Fibroadenoma	Found Dead	1.0%	0.0%		
	Killed Terminally			0.0%	0.0%
	Total	0.5%	0.0%		
Parathyroid Adenoma	Found Dead	3.2%	1.8%		
	Killed Terminally	11.5%	8.1%		
	Total	7.2%	4.3%		
Pituitary Adenoma	Found Dead	62.1%	40.0%†		
	Killed Terminally	62.7%	42.2%*		
	Total	62.4%	41.0%†		
Skin, Fibroma	Found Dead			3.1%	3.6%
	Killed Terminally	5.9%	2.3%		
	Total	4.5%	3.0%		
Skin, Papilloma	Found Dead	10.2%	0.0%†		
	Killed Terminally	5.9%	4.5%		
	Total	8.0%	2.0%*		
Testes, Interstitial Tumor	Found Dead	2.0%	0.0%		
	Killed Terminally			5.9%	17.8%*
	Total			4.0%	8.0%
Thyroid, Follicular Cell Adenoma	Found Dead	1.0%	0.0%		
	Killed Terminally	3.9%	0.0%		
	Total	2.5%	0.0%		

*Comparison with control group is statistically significant ($p<0.05$).
†Comparison with control group is statistically significant ($p<0.01$).

TABLE 4

EFFECT OF IBOPAMINE ON THE OCCURRENCE OF BENIGN LESIONS IN FEMALE RATS

Lesion	Group	Decreased Incidence Ibopamine (mg/kg/day) 0	Decreased Incidence Ibopamine (mg/kg/day) 180	Equal or Increased Incidence Ibopamine (mg/kg/day) 0	Equal or Increased Incidence Ibopamine (mg/kg/day) 180
Adrenal Cortical Adenoma	Found Dead	32.4%	19.6%		
	Killed Terminally	39.3%	18.4%†		
	Total	35.5%	19.0%†		
Mammary Gland Adenoma	Found Dead			27.0%	37.3%
	Killed Terminally	41.6%	8.2%‡		
	Total	33.5%	23.0%*		
Mammary Gland Fibroadenoma	Found Dead	12.6%	9.8%		
	Killed Terminally			9.0%	10.2%
	Total	11.0%	10.0%		
Parathyroid Adenoma	Found Dead	3.2%	0.0%		
	Killed Terminally	4.4%	0.0%		
	Total	3.7%	0.0%		
Pituitary Adenoma	Found Dead	87.4%	82.4%		
	Killed Terminally	74.2%	67.3%		
	Total	81.5%	75.0%		
Skin, Fibroma	Found Dead	1.8%	0.0%		
	Killed Terminally	3.4%	2.0%		
	Total	2.5%	1.0%		
Skin, Papilloma	Found Dead	0.9%	0.0%		
	Killed Terminally			1.1%	2.0%
	Total			1.0%	1.0%
Thyroid, Follicular Cell Adenoma	Found Dead			0.0%	0.0%
	Killed Terminally			1.1%	1.0%
	Total			0.5%	1.0%

*Comparison with control group is statistically significant ($p<0.05$).
†Comparison with control group is statistically significant ($p<0.01$).
‡Comparison with control group is statistically significant ($p<0.001$).

cific lesion was significantly different between control and treated groups. In other words, each lesion was simply listed as benign or malignant, and the number of lesions were noted for each animal. Then the total number of lesions were compared statistically and reported in Table 2. The lists of specific lesions from which these data were compiled are presented in Tables 3 through 6.

Benign neoplasms that were found in male and female rats are presented in Tables 3 and 4, respectively. The benign lesions are listed as being increased or decreased as a correlate of ibopamine treatment (180 mg/kg/day). Ibopamine-treated rats of both sexes had fewer benign lesions than controls, with reductions in adrenal cortical adenoma, pituitary adenoma, mammary gland adenoma and skin papilloma reaching statistical significance in at least one sex ($p<0.05$, at least). However, as also seen in Table 3, interstitial cell tumor of the testes occurred more frequently ($p<0.05$) in ibopamine-treated rats that were killed terminally than in their corresponding controls.

Malignant lesions observed in male and female rats are presented in Tables 5 and 6, respectively. The incidence of adrenal pheochromocytoma was significantly lower ($p<0.05$, at least) in all ibopamine-treated male rats and in females that died of other causes during the study. In addition, mammary gland adenocarcinoma was significantly reduced ($p<0.001$) in treated females, occurring in 15.5% of controls versus 1.0% of ibopamine-treated rats (Table 6). Although the incidences of certain other malignancies such as hepatocellular carcinoma, pancreatic islet cell carcinoma, and adrenal cortical carcinoma were greater in controls than in ibopamine-treated rats, the differences did not reach statistical significance. Squamous cell skin carcinoma was the only malignant lesion that occurred more often ($p<0.05$) in ibopamine-treated rats. The higher incidence was sex-linked, with the lesion occurring in 3% of drug-treated females compared with 0% of controls.

The frequencies of 6 neoplastic lesions were inversely related to dose in ibopamine-treated rats. As seen in Table 7, statistically significant, dose-related trends were obtained in males for adrenal cortical adenoma, pituitary adenoma, and adrenal pheochromocytoma. Similar effects occurred in females for adrenal cortical adenoma, mammary gland adenoma and mammary gland adenocarcinoma. Skin papilloma and adrenal pheochromocytoma were also significantly

TABLE 5

EFFECT OF IBOPAMINE ON THE OCCURRENCE OF MALIGNANT LESIONS IN MALE RATS

Lesion	Group	Decreased Incidence Ibopamine (mg/kg/day) 0	Decreased Incidence Ibopamine (mg/kg/day) 180	Equal or Increased Incidence Ibopamine (mg/kg/day) 0	Equal or Increased Incidence Ibopamine (mg/kg/day) 180
Adrenal Cortical Carcinoma	Found Dead	3.1%	1.8%		
	Killed Terminally	2.9%	2.2%		
	Total	3.0%	2.0%		
Adrenal Pheochromocytoma	Found Dead	21.4%	0.0*		
	Killed Terminally	23.5%	15.6%		
	Total	22.5%	7.0*		
Brain, Astrocytoma	Found Dead	3.1%	1.8%		
	Killed Terminally			0.0%	2.2%
	Total			1.5%	2.0%
Brain, Oligodendroglioma	Found Dead	2.0%	0.0%		
	Killed Terminally	1.0%	0.0%		
	Total	1.5%	0.0%		
Jejunum, Adenocarcinoma	Found Dead	2.0%	0.0%		
	Killed Terminally			0.0%	0.0%
	Total	1.0%	0.0%		
Renal Adenocarcinoma	Found Dead	1.0%	0.0%		
	Killed Terminally			1.0%	2.2%
	Total			1.0%	1.0%
Hepatocellular Carcinoma	Found Dead	4.1%	1.8%		
	Killed Terminally	6.9%	2.2%		
	Total	5.5%	2.0%		
Lung, Broncheolar-Alveolar Carcinoma	Found Dead	1.0%	0.0%		
	Killed Terminally	1.0%	0.0%		
	Total	1.0%	0.0%		
Mammary Gland Adenocarcinoma	Found Dead	2.0%	1.8%		
	Killed Terminally	2.9%	0.0%		
	Total	2.5%	1.0%		
Pancreas, Islet Cell Carcinoma	Found Dead	7.1%	1.9%		
	Killed Terminally	7.8%	4.4%		
	Total	7.5%	3.0%		
Skin, Basal Cell Tumor	Found Dead			0.0%	0.0%
	Killed Terminally	3.0%	2.3%		
	Total	1.5%	1.0%		
Stomach, Squamous Cell Carcinoma	Found Dead	1.0%	0.0%		
	Killed Terminally			0.0%	0.0%
	Total	0.5%	0.0%		
Thyroid, C-Cell Carcinoma	Found Dead	7.1%	3.6%		
	Killed Terminally	7.8%	6.7%		
	Total	7.5%	5.0%		
Thyroid, Follicular Cell Carcinoma	Found Dead	1.0%	0.0%		
	Killed Terminally			1.0%	2.2%
	Total			1.0%	1.0%

*Comparison with control group is statistically significant ($p<0.001$).

TABLE 6

EFFECT OF IBOPAMINE ON THE OCCURRENCE OF MALIGNANT LESIONS IN FEMALE RATS

Lesion	Group	Decreased Incidence Ibopamine (mg/kg/day) 0	Decreased Incidence Ibopamine (mg/kg/day) 180	Equal or Increased Incidence Ibopamine (mg/kg/day) 0	Equal or Increased Incidence Ibopamine (mg/kg/day) 180
Adrenal Cortical Carcinoma	Found Dead			0.0%	0.0%
	Killed Terminally	5.6%	0.0%		
	Total	2.5%	0.0%		
Adrenal Pheochromocytoma	Found Dead	9.0%	0.0%		
	Killed Terminally			1.1%	6.1%
	Total	5.5%	3.0%		
Brain, Astrocytoma	Found Dead			0.0%	0.0%
	Killed Terminally	2.2%	0.0%		
	Total	1.0%	0.0%		
Brain, Granular Cell Tumor	Found Dead			0.0%	0.0%
	Killed Terminally			0.0%	2.0%
	Total			0.0%	1.0%
Duodenum, Adenocarcinoma	Found Dead			0.0%	2.0%
	Killed Terminally			0.0%	0.0%
	Total			0.0%	1.0%
Renal Transitional Cell Carcinoma	Found Dead	0.9%	0.0%		
	Killed Terminally			0.0%	0.0%
	Total	0.5%	0.0%		
Liver, Cholangiocarcinoma	Found Dead	0.9%	0.0%		
	Killed Terminally			0.0%	2.0%
	Total			0.5%	1.0%
Mammary Gland Adenocarcinoma	Found Dead	15.3%	0.0%†		
	Killed Terminally	15.7%	2.0%†		
	Total	15.5%	1.0%‡		
Pancreas, Islet Cell Carcinoma	Found Dead			1.8%	3.9%
	Killed Terminally	4.5%	2.0%		
	Total			3.0%	3.0%
Skin, Squamous Cell Carcinoma	Found Dead			0.0%	3.9%
	Killed Terminally			0.0%	2.0%
	Total			0.0%	3.0%
Skin, Basal Cell Tumor	Found Dead			0.0%	0.0%
	Killed Terminally	1.1%	0.0%		
	Total	0.5%	0.0%		
Thyroid, C-Cell Carcinoma	Found Dead	4.5%	2.0%		
	Killed Terminally			7.9%	14.3%
	Total			6.0%	8.0%
Thyroid, Follicular Cell Carcinoma	Found Dead			0.0%	0.0%
	Killed Terminally	2.2%	2.0%		
	Total			1.0%	1.0%
Uterus, Adenocarcinoma	Found Dead			0.0%	2.0%
	Killed Terminally			0.0%	2.0%
	Total			0.0%	1.0%

*Comparison with control group is statistically significant ($p<0.05$).
†Comparison with control group is statistically significant ($p<0.01$).
‡Comparison with control group is statistically significant ($p<0.001$).

TABLE 7

DOSE RELATED EFFECTS OF IBOPAMINE ON NEOPLASTIC LESION IN RATS

		Males Ibopamine (mg/kg/day)				Females Ibopamine (mg/kg/day)			
	Group	0	30	90	180	0	30	90	180
Benign Lesions									
Adrenal Cortical Adenoma	Found Dead	23.5%	17.4%	6.4%	5.5%‡	32.4%	28.8%	18.3%	19.6%*
	Killed Terminally	26.5%	24.5%	15.4%	15.6%	39.3%	22.0%	27.5%	18.4%*
	Total	25.0%	21.2%	11.1%	10.0%‡	35.5%	26.0%	22.0%	19.0%‡
Mammary Gland Adenoma	Found Dead	1.0%	0.0%	0.0%	0.0%	27.0%	16.9%	23.3%	37.3%
	Killed Terminally	0.0%	33.3%	33.3%	0.0%	41.6%	59.3%	36.8%	8.2%‡
	Total	0.5%	2.0%	2.0%	0.0%	33.5%	30.2%	26.6%	23.0%*
Pituitary Adenoma	Found Dead	62.1%	73.9%	45.8%	40.0%†	87.4%	76.3%	88.3%	82.4%
	Killed Terminally	62.7%	92.6%	87.0%	42.2%	74.2%	96.6%	100.0%	67.3%
	Total	62.4%	80.8%	59.2%	41.0%‡	81.5%	83.0%	91.7%	75.0%
Skin, Papilloma	Found Dead	10.2%	4.3%	4.3%	0.0%†	0.9%	0.0%	0.0%	0.0%
	Killed Terminally	5.9%	8.3%	8.5%	4.5%	1.1%	3.2%	4.5%	2.0%
	Total	8.0%	6.4%	6.4%	2.0%	1.0%	1.1%	1.2%	1.0%
Malignant Lesions									
Adrenal Pheochromocytoma	Found Dead	21.4%	10.9%	6.4%	0.0%‡	9.0%	0.0%	1.7%	0.0%†
	Killed Terminally	23.5%	11.3%	19.2%	15.6%	1.1%	2.4%	5.0%	6.1%
	Total	22.5%	11.1%	13.1%	7.0%‡	5.5%	1.0%	3.0%	3.0%
Mammary Gland Adenocarcinoma	Found Dead	2.0%	2.2%	0.0%	1.8%	15.3%	15.3%	10.0%	0.0%†
	Killed Terminally	2.9%	0.0%	0.0%	0.0%	15.7%	22.2%	15.8%	2.0%*
	Total	2.5%	2.0%	0.0%	1.0%	15.5%	17.4%	11.4%	1.0%‡

*Trend is statistically significant ($p<0.05$).
†Trend is statistically significant ($p<0.01$).
‡Trend is statistically significant ($p<0.001$).

reduced compared to controls in dose-related fashion in ibopamine-treated males and females, respectively, that died during the course of this study.

Lower frequencies of nonneoplastic lesions in at least one sex of ibopamine-treated rats were also dose-related (Table 8). Significant ($p<0.05$, at least) trends leading to lower incidences of chronic glomerulonephropathy, renal pelvic mineralization, hepatocellular proliferative nodule, mammary galactoceles and chronic cardiomyopathy occurred. There were no dose-related trends to suggest that nonneoplastic diseases increased as a consequence of ibopamine treatment.

In addition to reducing disease, ibopamine retarded age-related atrophy of the adrenal cortex. The adrenal cortex of the rat undergoes severe atrophy by two years of age [17]. However, the zona glomerulosa of ibopamine-treated rats was appreciably thicker than the zona glomerulosa in age-matched controls (Fig. 2). This difference between control and ibopamine-treated rats was highly significant ($p<0.001$) and dose-related. In males dosed with 30, 90, and 180 mg/kg/day of ibopamine, atrophy of the zona glomerulosa was prevented in 20%, 34%, and 42% ($p<0.001$), respectively. In females receiving the same doses, atrophy of the zona glomerulosa was prevented in 9%, 19% and 37% ($p<0.001$), respectively. Since the zona glomerulosa from 24-month-old ibopamine-treated rats were comparable to those in 3-month old untreated rats, the data suggest that age-related, atrophic changes in the adrenal cortex were prevented by ibopamine.

Another atrophic change partially retarded by ibopamine was hair loss. As seen in Table 8, alopecia in male rats was significantly reduced in a dose-related fashion.

DISCUSSION

An initial concern with the results of this study was that the significantly lower incidences of lesions in ibopamine-treated rats might be an artifact of early death. Theoretically, if more ibopamine-treated rats than controls died before the age at which lesions occurred spontaneously, and if all rats were used for calculating the differences between groups, then a lower incidence of lesions in treated rats could be apparent but not real. Thus, when mortality is very different between groups, a time-adjusted analysis is required to determine if differences in lesion incidence are really due to treatment. In the present study, differences in mortality between control and ibopamine-treated rats were not statistically different, suggesting that the lower incidences of lesions in ibopamine-treated rats were drug-related. Support for this view also derives from the fact that differences in mortality and lesion incidences in the two control groups were not consistent with bias resulting from early deaths. For example, no male rats from control group I and 7 male rats from control group II were dead after 12 months on the study. However, adrenal cortical adenoma and pheochromocytoma occurred more often in control group II (29% and 24%, respectively) than in control group I (16% and 18%, respectively). If early deaths were skewing the incidence

TABLE 8

DOSE RELATED EFFECTS OF IBOPAMINE ON NONNEOPLASTIC LESIONS IN RATS

Lesion	Group	Males Ibopamine (mg/kg/day) 0	30	90	180	Females Ibopamine (mg/kg/day) 0	30	90	180
Alopecia	Found Dead	22.4%	26.1%	10.6%	9.1%*	12.7%	6.8%	11.7%	13.7%
	Killed Terminally	38.6%	39.6%	34.0%	11.4%†	20.2%	29.0%	18.2%	10.2%
	Total	30.7%	33.0%	22.3%	10.1%‡	16.1%	14.4%	13.4%	12.0%
Chronic Glomerulonephropathy	Found Dead	73.5%	89.1%	60.4%	65.5%	33.3%	20.3%	30.0%	13.7%*
	Killed Terminally	84.3%	75.0%	85.0%	84.4%	39.3%	45.5%	27.3%	30.6%
	Total	79.0%	84.8%	67.6%	74.0%	36.0%	24.3%	29.6%	22.0%*
Renal Pelvic Mineralization	Found Dead	10.2%	6.5%	6.2%	1.8%*	48.6%	54.2%	36.7%	23.5%†
	Killed Terminally	4.9%	10.0%	10.0%	2.2%	52.8%	36.4%	27.3%	8.2%‡
	Total	7.5%	7.6%	7.4%	2.0%	50.5%	51.4%	35.2%	16.0%‡
Hepatocellular Proliferative Nodule	Found Dead	5.1%	10.9%	6.2%	1.8%	13.5%	8.5%	1.7%	0.0%‡
	Killed Terminally	15.7%	9.1%	16.7%	11.1%	18.0%	11.1%	40.0%	8.2%
	Total	10.5%	10.1%	10.3%	6.0%	15.5%	9.1%	11.2%	4.0%†
Mammary Galactoceles	Found Dead	13.3%	13.0%	4.2%	1.8%†	27.9%	39.0%	23.3%	17.6%
	Killed Terminally	13.7%	100.0%	66.7%	4.4%	24.7%	44.4%	42.1%	12.2%
	Total	13.5%	18.4%	7.8%	3.0%†	26.5%	40.7%	27.8%	15.0%*
Cardiomyopathy, Chronic	Found Dead	37.8%	29.2%	25.0%	47.3%	24.3%	10.2%	11.7%	9.8%†
	Killed Terminally	64.7%	75.0%	100.0%	46.7%	30.3%	0.0%	33.3%	14.3%*
	Total	51.5%	35.7%	30.8%	47.0%	27.0%	10.2%	12.7%	12.0%‡

*Trend is statistically significant ($p<0.05$).
†Trend is statistically significant ($p<0.01$).
‡Trend is statistically significant ($p<0.001$).

data of these two neoplastic diseases, then males from control group I which began dying later, i.e., after thirteen months on study, should have had the greater number of lesions. Similarly, early death was not correlated with a lower incidence of renal pelvic mineralization in female control rats. Although mortality was initially higher in females from control group II than in females from control group I, the number of renal pelvic lesions was higher in control group II (53% vs. 43%). Since mortality in male and female rats receiving ibopamine (180 mg/kg/day) was comparable to controls, but adrenal cortical adenoma, pheochromocytoma and renal pelvic mineralization were significantly lower than controls, the data suggest that ibopamine was more influential than early death upon the incidence of spontaneous lesions in the present study.

Nutritional factors also have the potential to affect the results of a study in which animals are chronically exposed to chemical agents. For example, long-term restriction of food intake has been shown to depress body weight, reduce tumors and increase life span in rodents of both sexes [3, 4, 20, 22, 31]. Since nutrition can modulate the incidence of disease, Rao *et al.* [26] recently reviewed the data from several National Cancer Institute-National Toxicology Program (NCI-NTP) 2 year chemical carcinogenicity studies to determine if the results were in some way correlated with differences in body weight between treated and control groups. Using body weight as an indicator of caloric intake, they showed that 10–20% reductions in body weight correlated with significantly lower incidences of mammary and anterior pituitary adenomas. Malignant lesions did not show comparable correlations in the NCI-NTP studies that were reviewed.

In the present study body weight and food intake were carefully monitored to determine if the reduced incidences of malignant and nonneoplastic lesions were associated with caloric restriction. Food intake in ibopamine-treated rats was comparable to controls throughout the study, even though body weight was slightly lower in the treated animals. Since reduced food consumption was clearly not responsible for the lower body weight in ibopamine-treated rats, it is unlikely that the antitumorigenic effect of the drug had a nutritional basis. The lower body weights may instead reflect a leaner body mass resulting from muscle maintenance in preference to fat production. Alternatively, the weight differences may have resulted from the mild diuretic action of ibopamine which has been observed in experimental and clinical studies [11,15]. This action may, in fact, account for the lower body weight of ibopamine-treated rats in the present study since clinical administration of the drug has been associated with slight weight loss in man [23].

The most obvious mechanism by which ibopamine reduced the incidence of disease, and especially certain endocrine tumors, is by inhibition of prolactin release from the pituitary. Since ibopamine retards prolactin secretion [10], it may thereby reduce pituitary tumors, mammary cancer and galactoceles which are related to hypersecretion of the hormone. Lactotropes possess dopamine receptors whose activation retards prolactin secretion, so that the ability of ibopamine to reduce prolactin-dependent lesions may be due simply to its dopaminergic effect on the pitui-

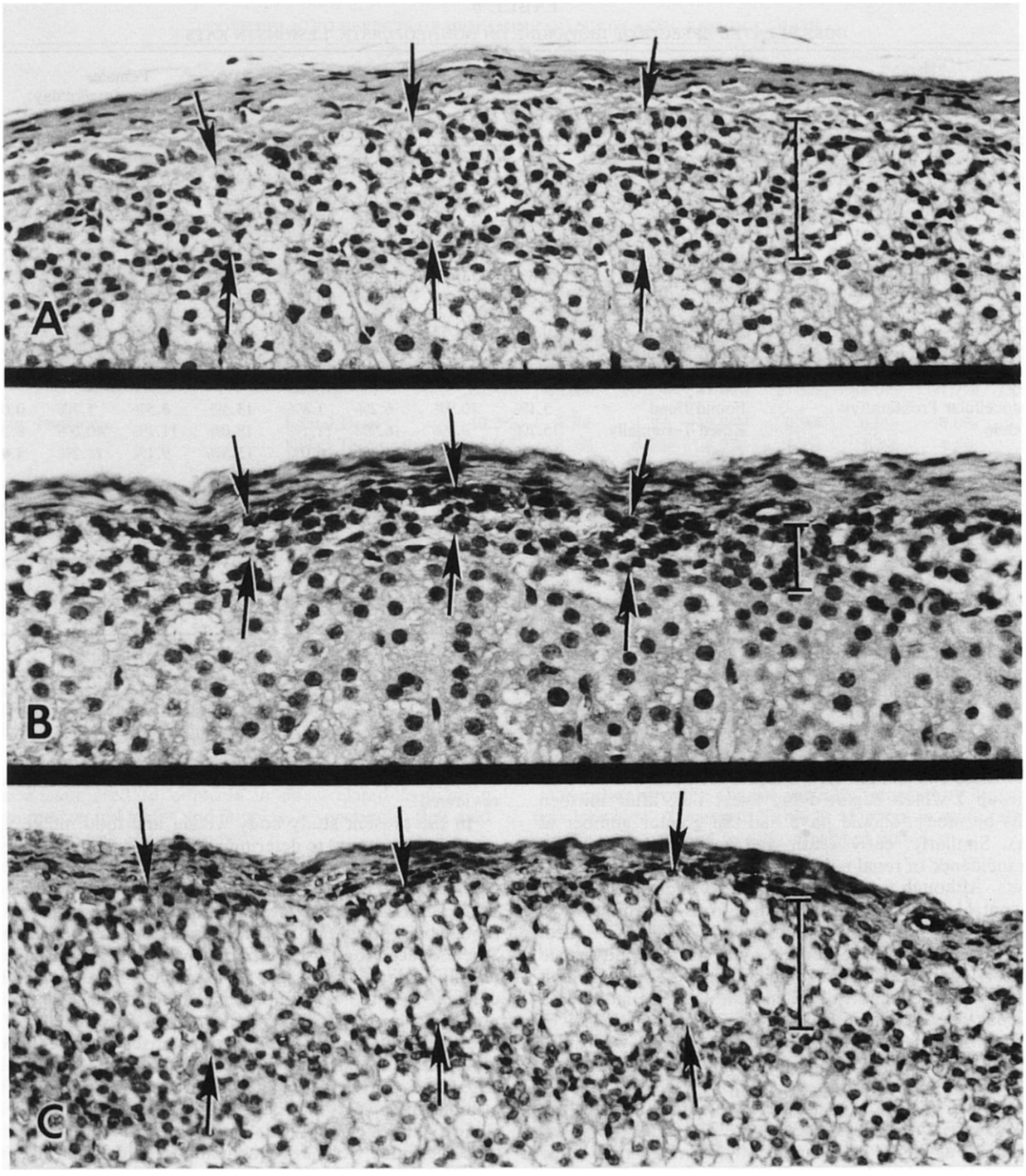

FIG. 2. Photomicrographs of outer adrenal cortex showing capsule, zona glomerulosa (arrows) and outer zona fasciculata. Approximate thickness of zona glomerulosa is indicated by a bar (I). (A) 3-month-old control rat. (B) Age-matched control, 2 years. (C) Ibopamine-treated for 2 years.

tary. The effectiveness of this therapeutic approach to treatment of diseases that are linked to hypersecretion of prolactin has been demonstrated [29].

However, only a portion of the lesions that were reduced by ibopamine are prolactin-dependent. Furthermore, clinical data suggest that the action of ibopamine on prolactin is more complex than one of simple suppression. For example, the drug significantly suppressed serum prolactin in man two hours after its initial administration, but baseline levels of the hormone were normal after eight days of repeated dosing [27]. Thus, rather than being chronically suppressed, prolactin secretion may be stabilized by continued adminis-

tration of ibopamine. Such action might retard the onset of prolactin-dependent tumors while still allowing sufficient hormone to provide positive support for other prolactin-dependent functions such as those of the immune system [24]. This possibility is supported by the fact that the lower incidences of adrenal cortical neoplasms, pheochromocytoma, chronic glomerulonephropathy, renal pelvic mineralization, hepatocellular proliferative nodule, chronic cardiomyopathy and retardation of adrenal cortical atrophy in ibopamine-treated rats are difficult to attribute to reduced prolactin secretion. While all of these effects cannot be explained by enhanced immune function either, the findings suggest that ibopamine has a more generalized mechanism of action than one simply involving inhibitory dopamine receptors in the pituitary.

The results of other carcinogenicity studies published by the National Toxicology Program and National Cancer Institute support the view that the prophylactic effects of ibopamine are related to its catecholaminergic properties. For example, the sympathomimetics ephedrine and phenylephrine increased survival and significantly reduced the incidence of leukemia and pheochromocytoma in rodents [5,16]. These effects were recently shown to be unrelated to changes in body weight [26]. In contrast, the catecholamine depleting drug reserpine increased the incidence of pheochromotoma and tumors of the testes and mammary glands [6]. Since ibopamine reduced a larger number of diseases than ephedrine or phenylephrine, the structural peculiarities of these drugs and their differential ability to activate catecholamine receptors, alone or in combination, may account for their unique prophylactic efficacies. Perhaps these putative structure:activity relationships can be enhanced, and new compounds developed for future study and/or prevention of certain intrinsic diseases of aging.

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REFERENCES

1. Armitage, P. Tests for linear trends in proportions and frequencies. *Biometrics* **11**: 375–386, 1955.
2. Arnsten, A. F. T. and P. S. Goldman-Rakic. α_2-adrenergic mechanisms in prefrontal cortex associated with cognitive decline in aged nonhuman primates. *Science* **230**: 1273–1276, 1985.
3. Barrows, C. H. and G. C. Kokkonen. Relationship between nutrition and aging. *Adv Nutr Res* **1**: 253–298, 1977.
4. Berg, B. N. and H. S. Simms. Nutrition and longevity in the rat II. Longevity and onset of disease with different levels of food intake. *J Nutr* **71**: 255–263, 1960.
5. Buckner, J. R. Toxicology and carcinogenesis studies of phenylephrine hydrochloride in F 344/N Rats and B6C3F1 Mice, CAS No. 61-76-7, Technical Report Series No. 322, National Toxicology Program, Research Triangle Park, NC, 1986.
6. Chu, K. C. Bioassay of reserpine for possible carcinogenicity, CAS No. 50-55-5, Technical Report Series No. 193, National Cancer Institute, Bethesda, 1982.
7. Cochran, W. G. Some methods for strengthening the common χ^2 test. *Biometrics* **10**: 417–451, 1954.
8. Cochran, W. G. and G. M. Cox. *Experimental Designs, 2nd Edition*. New York: John Wiley, 1957.
9. Cotzias, G. C., S. T. Miller, L. C. Tang, P. S. Papavasiliou and Y. Y. Wang. Levodopa, fertility and longevity. *Science* **196**: 549–550, 1977.
10. Ferrari, C., C. Barbieri, R. Caldera, P. Rampini, A. Paracchi, M. Boshen and W. G. Rauhe. Effects of ibopamine on serum prolactin and growth hormone levels in hyperprolactinemic and acromegalic subjects. *Clin Pharmacol Ther* **34**:74–78, 1983.
11. Ferrini, R. and G. Miragoli. Effect of ibopamine on diuresis and atrial blood pressure in anesthetized rata and its interactions with dopaminergic and α- and β-adrenergic agonists. *Arzneimittelforschung* **36**: 323–326, 1986.
12. Finch, C. E. Catecholamine metabolism in the brains of aging male mice. *Brain Res* **52**: 261–276, 1973.
13. Goldman-Rakic, P. S. and R. M. Brown. Regional changes of monoamines in cerebral cortex and subcortical structures of aging rhesus monkeys. *Neuroscience* **6**: 177–187, 1981.
14. Hoehn, M. M. and M. D. Yahr. Parkinsonism: onset, progression and mortality. *Neurology* **17**: 427–442, 1967.
15. Incerti, P. L., S. Badalamenti, E. Lorenzano, G. Graziani, A. Morganti, F. Salerno and P. Ghirardi. Humoral and renal effects of ibopamine in normal subjects. *Arzneimittelforschung* **36**: 405–407, 1986.
16. Irwin, R. Toxicology and carcinogenesis studies of ephedrine sulfate in F 344/N rats and B6C3F1 Mice, CAS No. 134-72-5, Technical Report Series No. 307, National Toxicology Program, Research Triangle Park, NC, 1986.
17. Jayne, E. P. Cytology of the adrenal gland of the rat at different ages. *Anat Res* **115**: 459–475, 1953.
18. Kendall, M. G. and A. Stuart. *The Advanced Theory of Statistics*, vol 2. New York: Hafner, 1967, pp. 549–553.
19. Markham, C. H., L. J. Treciokas and S. G. Diamond. Parkinson's disease and levodopa. *West J Med* **121**: 188–206, 1974.
20. McCay, C. M., M. F. Crowell and L. A. Maynard. The effect of retarded growth upon the length of life span and upon the ultimate body size. *J Nutr* **10**: 63–79, 1935.
21. Marshall, J. F. and N. Berrios. Movement disorders of aged rats: Reversal by dopamine receptor stimulation. *Science* **206**: 477–478, 1979.
22. Masaro, E. J., B. P. Yu, H. A. Bertrand and F. T. Lynd. Nutritional probe of the aging process. *Proc Fed Am Soc Exp Biol Med* **39**: 3178–3182, 1980.
23. Melloni, G. F., R. Melloni, G. M. Minoja, G. Scarazzati, G. C. Bruni, P. Loreti and R. Bauer. Clinical tolerability of ibopamine hydrochloride. *Eur J Clin Pharmacol* **19**: 409–411, 1981.
24. Nagy, E., I. Berczi and H. G. Friesen. Regulation of immunity in rats by lactogenic and growth hormones. *Acta Endocrinol* **102**: 351–357, 1983.
25. Peck, H. M. Design of experiments to detect carcinogenic effects of drugs. In: *Carcinogenesis Testing of Chemicals*, edited by L. Goldberg. Cleveland: CRC Press, Inc., 1974, pp. 1–14.
26. Rao, G. N., W. W. Piegorsch and J. K. Haseman. Influence of body weight on the incidence of spontaneous tumors in rats and mice of long term studies. *Am J Clin Nutr* **45**: 252–260, 1987.
27. Scarazzati, E., G. Catenazzo, C. F. Melloni, P. Ghirardi and L. Bianco. A preliminary evaluation of the effects of ibopamine on prolactin secretion. *Boll Soc Ital Biol Sper* **59**: 1631–1635, 1983.
28. Snedecor, G. W. and W. G. Cochran. *Statistical Methods*, 6th edition. Ames, IA: Iowa State University Press, 1976, pp. 246–260.
29. Sobrinko, L. G., M. C. Nunes, C. Calhaz-Jorge, J. C. Mauricio and M. S. Santos. Effect of treatment with bromocriptine on the size and activity of prolactin producing pituitary tumors. *Acta Endocrinol* **96**: 24–29, 1981.
30. Sweet, R. D. and F. A. McDowell. Five year's treatment of Parkinson's disease with levodopa. *Ann Int Med* **83**: 456–463, 1975.
31. Tucker, M. J. The effect of long term food restriction on tumors in rodents. *Int J Cancer* **23**: 803–807, 1979.
32. Winbland, B., R. Adolfsson, A. Carlsson and C. G. Gotfries. Alzheimers disease: a report of progress. In: *Aging*, vol 19, edited by S. Corkin. New York: Raven Press, 1982, pp. 26–29.