EC_{50}-values and maximum relaxations (E_{Am}) calculated from concentration-response curves plotted for apomorphine in middle cerebral and central ear arteries from rabbits

Pretreatment	Middle cerebral arteries			Central ear arteries		
	n	EC_{50} (moles · l^{-1})	E_{Am} (%)*	n	EC_{50} (moles · l^{-1})	E_{Am} (%)*
Control	7	$(3.6\pm1.1)\times10^{-7}$	58 ± 8	7	Contraction	
Methysergide 2.8×10^{-6} moles · l^{-1}	7	$(1.7\pm0.7)\times10^{-6}$	70 ± 7	6	$(3.4\pm0.6)\times10^{-5}$	91 ± 3
Phenoxybenzamine 3×10^{-5} moles · l^{-1}	12	$(2.3\pm0.9)\times10^{-7}$ [a]	84 ± 6 [b]	6	$(3.5\pm1.0)\times10^{-6}$ [c]	69 ± 5 [d]
Methysergide 2.8×10^{-6} moles · l^{-1} + phenoxybenzamine 3×10^{-5} moles · l^{-1}	7	$(8.0\pm3.7)\times10^{-7}$	84 ± 5 [b]	6	$(1.4\pm0.5)\times10^{-5}$ [a]	94 ± 2 [e]

Results are expressed as mean ± SE. [a] $p<0.05$ (comparison with methysergide). [b] $p<0.05$ (comparison with control); [c] $p<0.001$ (comparison with methysergide); [d] $p<0.01$ (comparison with methysergide); [e] $p<0.001$ (comparison with phenoxybenzamine). * E_{Am} is expressed as percentage of the maximum papaverine (10^{-4} moles · l^{-1}) relaxation.

(5%) mixture. Flow rate was constant during each experiment. Perfusion pressure was monitored with a pressure transducer connected to a potentiometric recorder. All drugs were introduced into the perfusion reservoir. Paired arteries from the same animal were tested in the same organ bath. After equilibration for 40 min and treatment with high K^+ solution (30 mmoles · l^{-1} in middle cerebral arteries and 100 mmoles · l^{-1} in central ear arteries) to induce a steady contraction in order to reveal clear-cut dilatory responses, cumulative concentration-response curves were run with apomorphine (concentrations in the medium from 3×10^{-8} moles · l^{-1} to 10^{-4} moles · l^{-1}). Apomorphine doses were added before treatment (control curve) and after introduction of the drugs: phenoxybenzamine (3×10^{-5} moles · l^{-1}) methysergide (2.8×10^{-6} moles · l^{-1}) or phenoxybenzamine plus methysergide in the same concentrations as above. These concentrations were chosen according to the results of previous studies[3,5]. These drugs were applied to the vessels for 20 min and remained in the perfusion fluid while apomorphine was added. At the end of each experiment, papaverine was introduced into the perfusion fluid (10^{-4} moles · l^{-1}) as an arbitrary reference. The relaxant response of apomorphine was characterized by the median effective concentration (EC_{50}) and the maximum response (E_{Am}) established from the concentration-response curves. The E_{Am} was expressed as a percentage of the maximum relaxation induced by papaverine. Statistical comparisons were made using Student's t-test.

Results and discussion. After an active tone was given with K^+ solution, apomorphine induced a concentration-dependent relaxation in middle cerebral arteries but produced a slight contractile effect in central ear arteries. Methysergide did not influence the vasodilator action of apomorphine in middle cerebral arteries, either in the absence or in the presence of phenoxybenzamide. EC_{50} and E_{Am} were similar in each case (table). In central ear arteries, apomorphine induced a contractile response which was reversed to dilatation after treatment with phenoxybenzamine or methysergide. As shown in the table, the maximum relaxation due to apomorphine (E_{Am}) was higher with methysergide than with phenoxybenzamine, but its affinity was lower. Moreover the comparison of EC_{50} values indicated that apomorphine had a higher relaxant potency in middle cerebral arteries than in central ear arteries after pretreatment with methysergide, phenoxybenzamine or methysergide plus phenoxybenzamine.

Our results suggest that the relaxant effect of apomorphine in rabbit middle cerebral arteries does not implicate 5-HT receptors. On the contrary, the apomorphine response in central ear arteries seems to involve the activation of 5-HT receptors, given that methysergide, a competitive 5-HT receptor antagonist in peripheral arteries[5], was able to potentiate apomorphine relaxation.

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Effects of fluoride on glycosaminoglycan of cancellous and cortical bone of rabbits

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Summary. The present report deals with the effect of excessive ingestion of fluoride on glycosaminoglycan (GAG). Increase in fluoride deposition in bone, and in circulating levels of fluoride in serum, are also reported. Among the 3 constituents of GAG investigated; hexosamine, uronic acid and sulphate, the content remained unaltered except for sulphate.

It is reasonably well established that fluoride poisoning affects the structure and normal functioning of both osseous and non-osseous tissues[2-7]. The mineralization process in skeletal tissues has been reported to be defective in fluoride poisoning[8,9]. One of the possible explanations for defective mineralization in bone is that the organic matrix of bone might be abnormal. Indirect evidence obtained through autoradiographic (using S^{35}) and other histochemical studies suggest changes in glycosaminoglycans (GAG) in bone and tooth during mineralization[10-12].

The present investigation was undertaken with the objective of examining the effect of fluoride on cortical and cancellous bones with reference to GAG content, and their chemical composition.

Rabbits aged 2 months were fed 10 mg of sodium fluoride per kg b.wt daily via the intragastric route for periods of up to 10 months. The rabbits were killed after varying periods (6–10 months) of fluoride treatment. The cortical diaphyseal bone from the femur, and cancellous bone from the iliac crest region were dissected out and cleaned from marrow. Fat free bone powder was prepared using an ether-acetone mixture (1:1) and acetone. The bone was ground to a fine powder by using a milling machine with xylene as a cooling fluid, which kept the temperature below 25 °C. The powder was washed with xylene, acetone and ether and was dried at room temperature[13]. 1 g of bone powder, both from cortical and from cancellous bones, was suspended in 40 ml of digestion mixture containing 0.005 M cystein hydrochloride and 0.2 M EDTA for simultaneous demineralization. An aqueous solution of papain (0.1 ml/40 ml of digestion mixture) was also added. The enzyme papain contained 1.7 mg of protein (10–15 units/mg protein). The GAG released after demineralization and proteolytic enzyme digestion was precipitated with cetylpyridinium chloride (CPC) as described previously[13]. Chemical analysis of uronic acid[14] and hexosamines[15], and differential assay of glucosamines and galactosamines[16] and sulphate[17] were carried out. Fluoride in bone[18] and serum[19] was determined using a PHM 84 Research pH meter (Radiometer, Copenhagen) with a fluoride specific electrode.

Tissues obtained from normal rabbits aged 8–10 months maintained under the same laboratory conditions but not given sodium fluoride were subjected to the same treatment for data on control animals.

Effects of fluoride ingestion on fluoride deposition in cortical and cancellous bones and on levels of fluoride in the serum are reported in table 1. The data show that the defatted normal dry bone had 805 and 895 ppm of fluoride for cortical and cancellous bones respectively. Daily ingestion of 10 mg NaF/kg weight for a period of 6 months substantially increased the fluoride content of both cortical and cancellous bones. Of the 2 bones, the cancellous bone had a greater affinity for fluoride deposition than compact bone. Besides, the fluoride content of cancellous bone increased with fluoride ingestion whereas, in the cortical bone, a similar increase was not detected. During fluoride ingestion the serum fluoride content also increased from 0.07 to 0.5 ppm over a period of 10 months.

In tables 2a and 2b, the results obtained on CPC precipitable GAG and their chemical composition in both cortical and cancellous bones are reported. Among the 3 constituents of CPC precipitable GAG which have been investigated; hexosamine, uronic acid and sulphate, the content remained unaltered except for sulphate. The sulphate content of both cortical and cancellous bones increased significantly ($p < 0.0005$).

The molar ratio of uronic acid to hexosamine is constant but the molar ratio of sulphate to hexosamine increased

Table 1. Fluoride content of bone* and serum** of normal rabbits and rabbits treated with sodium fluoride

	Cortical bone Mean ± SD	Cancellous bone Mean ± SD	Serum Mean ± SD
Control	805 ± 43.7	895 ± 57.7	0.07 ± 0.02
6 months	3873 ± 468.2	5870 (2)	0.36 ± 0.08
8 months	3817 ± 407.2	7467 ± 550.8	0.45 ± 0.06
10 months	3700 ± 264.6	7550 ± 477	0.50 ± 0.01

* Data expressed as ppm of fluoride in dry defatted bone. ** Data expressed as ppm of fluoride in serum. The number of animals are 3 except in one experiment where 2 animals have been used. The number has therefore been indicated in parentheses. ± SD = standard deviation. p-value for bone and serum fluoride is $p < 0.005$.

Table 2a. Chemical analysis of CPC precipitable GAG from cancellous bone of normal rabbits and rabbits treated with sodium fluoride

	Total hexosamines Mean ± SD	Galactosamines Mean ± SD	Glucosamines Mean ± SD	Uronic acid Mean ± SD	Sulphate Mean ± SD	Molar ratios** Uronic acid:hexosamine	Sulphate:hexosamine	Ratios Galactosamines:glucosamines
Control (3)	21.2 ± 1.1	56.3 ± 2.5	43.7 ± 2.5	23.8 ± 0.5	17.5 ± 0.5	1.04	1.53	1.29
6 months (2)	23.7	65.0	35.0	25.4	28.5*	0.99	2.24	1.86
8 months (3)	23.7 ± 1.7	67.3 ± 4.2	32.7 ± 4.2	24.9 ± 1.6	28.5* ± 1.3	0.97	2.24	2.06
10 months (3)	23.1 ± 1.0	65.0 ± 4.4	35.0 ± 4.4	23.9 ± 1.2	28.8* ± 0.8	0.96	2.32	1.86

* Significant at $p < 0.0005$. ** Molar ratios are based on hexosamine = 1.0. Data expressed as mg% of dry defatted bone for CPC precipitable GAG. Data expressed as mg% of dry CPC precipitable GAG for total hexosamine, uronic acid and sulphate. Data expressed as % of total hexosamines for galactosamine and glucosamine. The number of experiments is indicated in parentheses. ± SD = standard deviation.

Table 2b. Chemical analysis of CPC precipitable GAG from cortical bone of normal rabbits and rabbits treated with sodium fluoride

	Total hexosamines Mean ± SD	Galactosamines Mean ± SD	Glucosamines Mean ± SD	Uronic acid Mean ± SD	Sulphate Mean ± SD	Molar ratios** Uronic acid:hexosamine	Sulphate:hexosamine	Ratios Galactosamines glucosamines
Control (3)	20.8 ± 0.7	57.7 ± 1.5	42.3 ± 1.5	22.8 ± 0.6	15.6 ± 0.5	1.01	1.39	1.36
6 months (3)	27.9 ± 0.4	64.0 ± 4.0	36.0 ± 4.0	29.5 ± 1.2	27.0* ± 1.0	0.98	1.79	1.78
8 months (3)	25.9 ± 0.5	66.0 ± 3.5	34.0 ± 3.5	26.2 ± 0.4	26.3* ± 0.6	0.95	1.91	1.94
10 months (3)	21.7 ± 2.9	59.3 ± 1.5	40.7 ± 1.5	25.7 ± 1.5	28.0* ± 1.0	1.09	2.40	1.46

* Significant at $p < 0.0005$. ** Molar ratios are based on hexosamine = 1.0. Data expressed as mg% of dry defatted bone for CPC precipitable GAG. Data expressed as mg% of dry CPC precipitable GAG for total hexosamine, uronic acid and sulphate. Data expressed as % of total hexosamines for galactosamine and glucosamine. The number of experiments is indicated in parentheses. ± SD = standard deviation.

both in the cortical and cancellous bones. Similarly the ratio of galactosamine to glucosamine increased in both cortical and cancellous bones.

The increase in sulphate content of both cancellous and cortical bones may either be due to an increased rate of sulphation or due to increased sulphated GAG content. The increase in the molar ratio of sulphate to hexosamine and the increase in the ratio of galactosamine to glucosamine would suggest that in fluoride poisoning both enhanced sulphation and sulphated GAG formation are taking place. This report provides evidence that fluoride has pronounced effects on the chemical composition of CPC precipitable GAG, both in cortical and cancellous bones.

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Demonstration of vascular dopamine receptors in membranes from rabbit renal artery using ^{3}H-spiroperidol binding

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Summary. Binding of ^{3}H-spiroperidol to membranes branes from rabbit renal artery was found to be saturable and of high affinity. Dopamine receptor antagonists inhibited binding much more potently than α-adrenergic antagonists and dopamine was much more potent than noradrenaline, indicating that ^{3}H-spiroperidol labels vascular dopamine receptors in rabbit renal artery.

The third endogenous catecholamine - dopamine - differs from the other endogenous catecholamines in exerting vasodilation in the renal, mesenteric and coronary arterial vascular beds of the intact anesthetized dog[2], which can only be inhibited by dopamine receptor antagonists like haloperidol[3], bulbocapnine[4] and phenothiazines[5]. Demonstration of such antagonism led to the concept of the existence of a specific vascular dopamine receptor[6]. More recently, specific antagonism to dopamine-induced relaxation could be demonstrated in vitro on isolated blood vessels, thus further supporting this hypothesis[7-11]. Radio-ligand binding studies have been used during the past few years to identify adrenergic receptors directly at the molecular level[12]. With this technique we have been successful in demonstrating dopamine receptors in a membrane fraction from rabbit mesenteric artery using ^{3}H-spiroperidol as the ligand[13]. The aim of the present study was to find out whether or not dopamine receptors can also be identified on rabbit renal artery by the use of ^{3}H-spiroperidol binding.

Methods. Radio-ligand binding assay. Rabbits of either sex, weighing 1.8-2.5 kg, were killed by a blow on the head. The renal arteries were excised and cleaned of connective tissue. For each binding experiment 20-24 renal arteries (approximately 400 mg wet weight) were pooled. Membrane preparations and radio-ligand assays using ^{3}H-spiroperidol were performed exactly as recently described in rabbit mesenteric arteries[13]. Briefly: arteries were homogenized in ice-cold 0.25 M sucrose, containing 1 mM $MgCl_2$ and 5 mM Tris-HCl pH 7.4 and centrifuged at 1000 × g 15 min at 4 °C. The pellets were discarded and the supernatant centrifuged at 50,000 × g 25 min. The resulting pellets were washed twice with incubation buffer (50 mM Tris-HCl containing 120 mM NaCl, 5 mM KCl, 2 mM $MgCl_2$ pH 7.4) and finally resuspended in 2.6 ml of incubation buffer containing 10 µM pargyline and 0.1% ascorbic acid to give a final pH of 7.1 at 37 °C. For the binding assay the incubation mixture contained 100 µl membrane suspension, 50 µl of various concentrations of ^{3}H-spiroperidol (ranging from 1 to 40 nM for the saturation experiments) or of 5 nM ^{3}H-spiroperidol (for competition experiments) and 50 µl of H_2O or (+)-butaclamol (10 µM) or (for competition experiments) 50 µl of various concentrations of the competing agents.

Incubations were carried out for 15 min at 37 °C. Incubations were terminated by adding 1 ml of ice-cold incubation buffer to the entire incubation mixture followed by rapid filtration over Whatman GF/C filters. The filters were washed by 3 5-ml rinses with ice-cold 50 mM Tris-HCl buffer pH 7.7 within 25 sec. After drying (1 h at 95 °C) radioactivity bound to membranes was counted in a Triton X-100/toluene scintillation mixture at an efficiency of 42%. 'Nonspecific' binding of ^{3}H-spiroperidol was defined as radioactivity bound to membranes which is not displaced by a high concentration of (+)-butaclamol (10 µM). 'Specific' binding of ^{3}H-spiroperidol is defined as total radioac-