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-

tivity minus unspecific binding and amounted to 60% at 5 nM ^{3}H-spiroperidol.

Data analysis. The experimental data given in the figures and the table are means ± SEM of N experiments. The regression line was calculated by the least squares method. The equilibrium dissociation constant (K_D) and the maximal number of binding sites (B_{max}) were determined from plots according to Scatchard[14]. K_I-values for inhibition of specific ^{3}H-spiroperidol binding were determined as recently described[13]. All compounds used in the present study were from sources recently described[13].

Results and discussion. Specific binding of ^{3}H-spiroperidol to membranes derived from rabbit renal arteries increased with increasing ^{3}H-spiroperidol concentrations. Complete saturation was obtained at about 40 nM ^{3}H-spiroperidol (fig. 1). From the Scatchard-analysis[14] of these binding data a maximal number of binding sites (B_{max}) of 149 fmoles ^{3}H-spiroperidol bound/mg protein and a K_D-value of 15.9 ± 1.4 nM (N = 3) was calculated (fig. 1, inset). This K_D-value for ^{3}H-spiroperidol binding to rabbit renal artery is in good agreement with that recently determined on rabbit mesenteric artery (13.1 nM)[13]; it is, however, 10 times higher than the K_D-value for ^{3}H-spiroperidol binding to dopamine receptors in central nervous system[15] indicating that ^{3}H-spiroperidol may have a lower affinity to vascular than to central dopamine receptors.

The ^{3}H-spiroperidol binding sites in rabbit renal artery had typical characteristics of classical dopamine-receptors. Dopamine receptor antagonists inhibited binding much more potently than α-adrenoceptor antagonists (fig. 2). Similar results were obtained with the agonists: dopamine was 10 times more potent than noradrenaline; serotonin and isoprenaline were ineffective (table). Binding was stereospecific as shown by the 100 times greater potency of (+)-butaclamol than (−)-butaclamol in inhibiting binding (table). In addition, the K_I-value for spiroperidol (18 nM; table) was in good agreement with the K_D-value determined by equilibrium binding studies with ^{3}H-spiroperidol (15.9 nM; fig. 1) indicating the biological equivalence of ^{3}H-spiroperidol and unlabelled spiroperidol at the dopamine receptor.

Furthermore, the apparent affinities to the ^{3}H-spiroperidol binding sites in rabbit renal artery expressed as K_I-values for (+)-butaclamol, droperidol and metoclopramide were well comparable with their K_B-values for antagonism to dopamine-caused relaxation on isolated rabbit mesenteric arteries[10, 11] (table). The same holds true for dopamine: its K_I-value agreed well with its median effective concentration producing relaxation on isolated rabbit[7, 10, 11] and canine[8] arteries.

Thus in accordance with our recently reported data from rabbit mesenteric artery[13], ^{3}H-spiroperidol also seems to

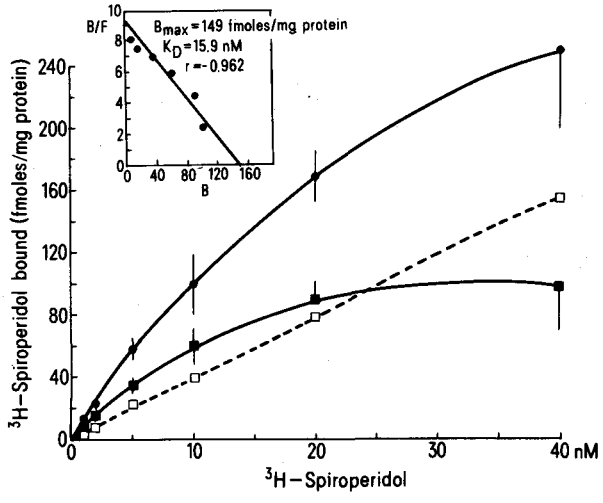

Figure 1. Binding of ^{3}H-spiroperidol to membranes from rabbit renal arteries as a function of increasing concentrations of ^{3}H-spiroperidol. Binding was carried out as described under 'methods' using ^{3}H-spiroperidol at various concentrations ranging from 1 to 40 nM in the absence (●—●) and presence (□-□) of 10 μM (+)-butaclamol for determination of specific binding (■—■). Given are means ± SEM of 3 experiments, performed in different membrane preparations. Inset: Scatchard-plot of specific ^{3}H-spiroperidol binding. The ratio B/F of specifically bound ^{3}H-spiroperidol (fmol/mg protein) to free ^{3}H-spiroperidol (nM) is plotted as function of B (= specifically bound ^{3}H-spiroperidol (fmol/mg protein)). Each value is the mean of 3 experiments.

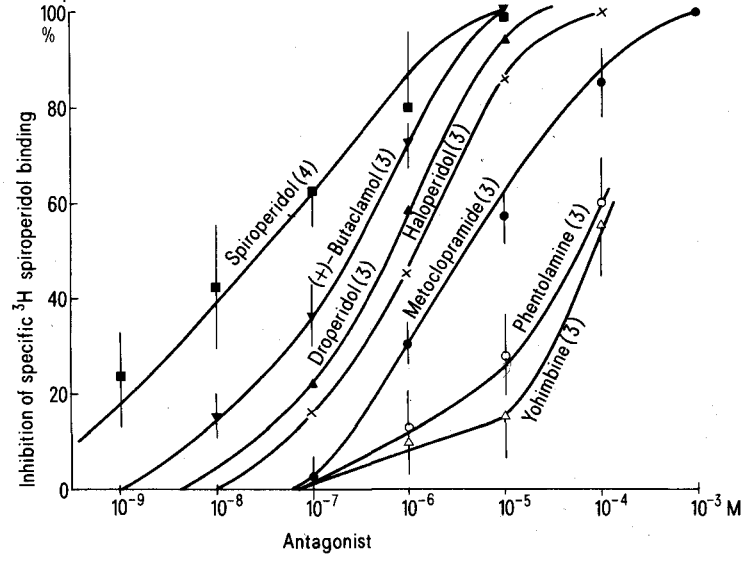

Figure 2. Inhibition of specific ^{3}H-spiroperidol binding to membranes from rabbit renal arteries by dopamine- and α-adrenoceptor-antagonists. Membranes were incubated with 5 nM ^{3}H-spiroperidol in the presence or absence of 4–5 concentrations of the antagonists and specific binding was determined[13]. '100% inhibition' refers to inhibition of binding evoked by 10 μM (+)-butaclamol. Given are means ± SEM. Number of experiments in parentheses.

Inhibition of specific ^{3}H-spiroperidol binding to membranes from rabbit renal artery by various agents

Antagonists	Inhibition of specific ^{3}H-spiroperidol binding K_I (μM)	Antagonism to dopamine-induced relaxation of isolated arteries K_B (μM)
Spiroperidol	0.018 ± 0.003 (4)	
(+)-Butaclamol	0.19 ± 0.015 (3)	0.12 (rabbit mesenteric artery[11])
Haloperidol	0.83 ± 0.076 (3)	
Droperidol	0.44 ± 0.05 (3)	0.9 (rabbit mesenteric artery[10])
Metoclopramide	4.18 ± 0.53 (3)	6.6 (rabbit mesenteric artery[10])
(−)-Butaclamol	17.4 ± 1.67 (3)	
Phentolamine	29.4 ± 3.42 (3)	
Yohimbine	45.2 ± 6.3 (3)	
(±)-Pindolol	> 1000 (3)	
Agonists and related compounds		**Relaxation of isolated arteries EC_{50} (μM)**
Dopamine	4.78 ± 0.68 (4)	7.0 (rabbit mesenteric artery[7,10,11]) 2.6 (canine renal artery[8])
Apomorphine	1.06 ± 0.08 (3)	125 (rabbit mesenteric artery[10])
(−)-Noradrenaline	58.33 ± 4.8 (3)	
(−)-Isoprenaline	> 1000 (3)	
Serotonin	> 1000 (3)	

The membrane fraction was incubated for 15 min at 37 °C with 5 nM ^{3}H-spiroperidol in the presence or absence of 4–5 different concentrations of the indicated agents. The IC_{50}-values were determined from concentration response-curves and converted into K_I-values as recently described[13]. Given are means ± SEM. Number of experiments in parentheses.
For comparison the median effective concentration (EC_{50}) for dopamine producing relaxation and the K_B-values (calculated from pA_2-values) for antagonism to dopamine-induced relaxation on isolated arteries are given from the literature. Tissue sources and references are shown in parentheses.

bind to a dopamine receptor in the rabbit renal artery. Accordingly, these results strongly support the view of the existence of vascular dopamine receptors on certain blood vessels.

Whether vascular dopamine receptors belong to the D_1-type (i.e. linked to adenylate cyclase) or to the D_2-type (i.e. not linked to adenylate cyclase) as defined by Kebabian and Calne[16] cannot be decided from the present experiments. However, the following facts favour the idea that vascular dopamine receptors may be tentatively classified as D_1-receptors: a) The order of potency for dopamine receptor antagonists, either used for inhibition of dopamine-evoked relaxation on the isolated rabbit mesenteric artery[10, 11] or used for inhibition of ^{3}H-spiroperidol binding to membranes from rabbit mesenteric[13] and renal artery (cf. fig. 2), is as follows; (+)-butaclamol > droperidol > metoclopramide. This order of potency is identical with that obtained for inhibition of stimulation of adenylate cyclase or ^{3}H-cis-flupenthixol binding to D_1-receptors in rat corpus striatum[17]. b) Dopamine produced relaxation on isolated arteries[7–11] and inhibited binding of ^{3}H-spiroperidol in rabbit mesenteric[13] and renal artery (cf. table) in micromolar concentrations. c) Apomorphine acts on the vascular dopamine receptor of the isolated rabbit mesenteric artery[10] and on canine renal artery[6] as a partial agonist. All of these facts fit very well into the concept of classifying the vascular dopamine receptor as a D_1-receptor[16], i.e. linked to adenylate cyclase.

This assumption is further supported by the recent demonstration of a dopamine-sensitive adenylate cyclase in homogenates of canine renal arteries[18], in a rat kidney particulate preparation[19] and in homogenates of rat glomerula[20]. Dopamine-induced stimulation of this adenylate cyclase could only be inhibited by dopamine receptor antagonists like haloperidol, spiroperidol and fluphenazine, whereas β-adrenoceptor antagonists like propranolol were without any effect. Taking these and the present results into consideration it seems to be justifiable to conclude that vascular dopamine receptors may be of the D_1-type (i.e. linked to adenylate cyclase).

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