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MODULATION OF DOPAMINE RECEPTORS BY CATIONS IN 7315a, MtTW15 AND ESTRADIOL-INDUCED PITUITARY TUMORS

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Abstract—Modulation of dopamine (DA) receptors by cations (Na^+, K^+, Mg^{2+}, Ca^{2+}) was compared in 7315a, MtTW15, and estradiol valerate-induced (EV-T) pituitary tumors, and intact adenohypophysis. In 7315a tumors, the affinity of [^{3}H]spiperone binding measured at 25° remained unchanged in the presence of each cation individually or all these cations together (IONS) compared to the affinity obtained using a buffer without ions; the density (B_{max}) was not affected by monovalent cations or Mg^{2+} and was decreased by Ca^{2+} or IONS. When binding experiments were done at 37°, monovalent cations increased affinity whereas divalent cations or IONS did not modify it, and none of these cations affected B_{max} values. In MtTW15 tumors, the affinity of [^{3}H]spiperone binding measured at 25° was not changed by Na^+ or IONS and was decreased by K^+ or divalent cations; the density was decreased by K^+ and unchanged by all the other cations. When binding experiments were done at 37°, Na^+ increased the affinity, whereas all the other cations did not affect it; the density was unaffected by all the cations studied. In EV-T assayed at 37°, the affinity was increased by monovalent cations or Mg^{2+} and was unchanged by Ca^{2+}; monovalent cations did not affect the density of [^{3}H]spiperone binding and divalent cations increased it. In binding experiments performed at 25° and 37°, choline chloride did not change the affinity or the density of [^{3}H]spiperone binding to DA receptors in the three pituitary tumors investigated, suggesting that the effect of cations was specific and not due to differences in ionic strength. In the rat normal anterior pituitary, Na^+ increased the affinity of [^{3}H]spiperone for the DA receptors, whereas the affinity was unchanged by Ca^{2+}; the density of [^{3}H]spiperone binding was unaffected by these cations. Our results suggest that DA receptors in 7315a and MtTW15 tumors are regulated abnormally by sodium, potassium, magnesium and calcium. In contrast, DA receptors in EV-T are regulated normally by monovalent cations and abnormally by divalent cations as compared to these receptors in intact pituitary tissue.

Sodium, potassium and magnesium ions have been shown to modulate dopamine (DA) receptors in intact porcine pituitary [1–3]. The DA receptors in prolactin (PRL)-secreting 7315a and MtTW15 pituitary tumors are apparently normal, although DA agonists do not inhibit PRL secretion [4, 5]. However, 7315a tumors have been shown to have abnormal calcium metabolism under basal conditions [6], but normal DA receptor regulation by sodium ion [7]. In contrast, sodium regulation of DA receptor antagonist binding sites in MtTW15 tumor is abnormal, [^{3}H]spiperone binding affinity being unaffected by sodium at 25° while in intact tissue sodium increases affinity at this temperature [8]. No data are yet available on 7315a and MtTW15 tumor regulation by potassium, magnesium and calcium ions. Rat estradiol valerate-induced pituitary tumors (EV-T) have been characterized as a model for human prolactinomas [9] and were found to have normal pituitary DA receptors [10, 11]; however no data are available on their ionic regulation. The aim of the present study was to investigate a possible defect in the modulation of the DA receptors in 7315a, MtTW15 and EV-T tumors by the cations sodium, potassium, magnesium and calcium. We have also studied for comparison the regulation of the DA receptors by sodium and calcium in the normal anterior pituitary.

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MATERIALS AND METHODS

Animals. Adult female Buffalo or Wistar–Furth rats weighing 175–200 g were used for experiments in 7315a and MtTW15 tumors, whereas adult female Sprague–Dawley rats (Charles River CD strain) weighing 200–250 g were used for studies in EV-T and intact anterior pituitary. All rats were housed two per cage under controlled temperature (22–23°) and light (monitored light–dark cycles with lights on from 5:00 a.m. to 7:00 p.m.), and received rat chow and water *ad lib*.

Buffalo and Wistar–Furth rats were inoculated s.c. under the back skin with 0.4 to 0.6 mL of a suspension of 7315a or MtTW15 tumors respectively. The 7315a and MtTW15 tumor suspensions were prepared as we described previously [7, 8]. Tumors were left to grow for about 3–4 weeks before killing the animals by decapitation. Then tumors were removed quickly, frozen immediately, and kept at −90° until assayed.

EV-T were induced for 30 weeks as we described

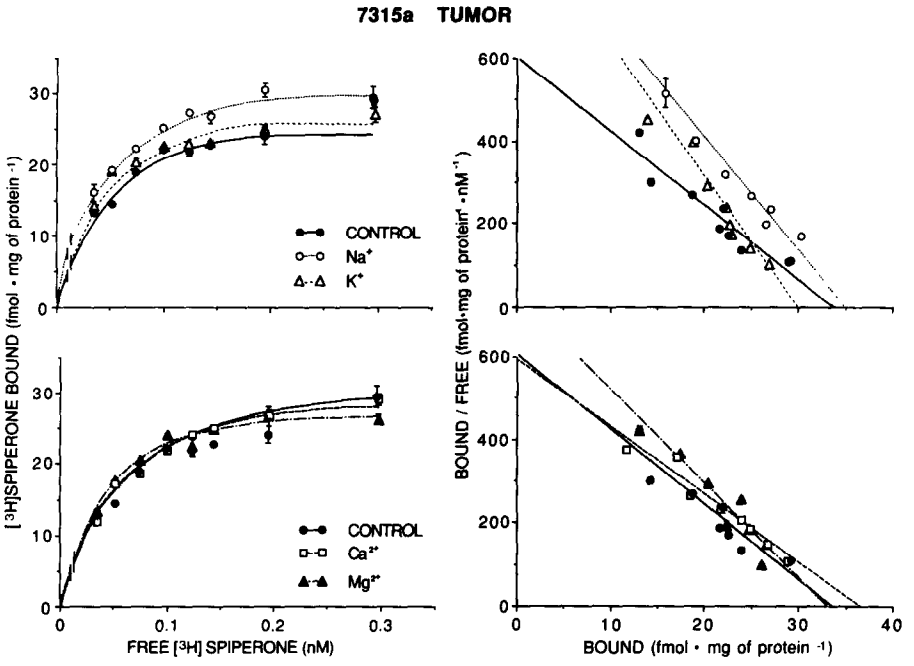

Fig. 1. Representative saturation experiments showing the effects of cations on [^{3}H]spiperone binding to DA receptors in 7315a tumors assayed at 37°. The concentrations of the cations were: 100 mM NaCl or 100 mM KCl as well as 10 mM $MgCl_2$ or 10 mM $CaCl_2$. The dissociation constant (K_D) values were 0.057 ± 0.009, 0.039 ± 0.004, 0.034 ± 0.004, 0.046 ± 0.006 and 0.062 ± 0.006 nM for the control, Na^+, K^+, Mg^{2+} and Ca^{2+} experiments respectively. The maximal binding capacity was, respectively, 34 ± 8, 35 ± 5, 30 ± 5, 33 ± 7, and 35 ± 5 fmol/mg protein. Values are means ± SE.

previously [10, 11]. Animals were killed by decapitation 1 week after their last estradiol valerate injection. The anterior lobe of the pituitary gland was dissected from the posterior lobe then frozen quickly and kept at −90° until assayed.

PRL determination. Trunk blood was collected into heparinized tubes, and plasma was separated by centrifugation at 4000 *g* for 10 min and kept at −20° until assayed for PRL by double-antibody radioimmunoassay as described previously [11].

Binding experiments. Tissue preparation for DA receptor assays was done at 0–4° essentially as before [7, 8, 10] with the following slight modification: the buffer composition was 0.25 M sucrose, 15 mM Tris–HCl, 100 μM EDTA, 12.5 μM nialamide and 0.01% ascorbic acid at pH 7.45.

The DA receptor antagonist binding site was investigated by saturation with increasing concentrations of [^{3}H]spiperone (7–8 concentrations, 0.02 to 0.45 nM); 2 μM (7315a and MtTW15 tumors) or 1 μM (EV-T and intact pituitary) (+)butaclamol dissolved in 0.01% ascorbic acid was used to estimate non-specific binding. Incubation was initiated by the addition of membrane suspension (0.2 mL containing an average of 0.2 mg of protein) to tubes containing 0.05 mL [^{3}H]spiperone (Amersham; 71.5 to 98.0 Ci/mmol), 0.05 mL (+)butaclamol or 0.05 mL of 0.01% ascorbic acid, and 1.7 mL (7315a and MtTW15 tumors) or 0.2 mL (EV-T and intact pituitary) of buffer: control buffer [15 mM Tris–HCl, 100 μM EDTA, 12.5 μM nialamide, 0.01% ascorbic acid, pH 7.45, at 0–4°], control buffers with cations [Na^+ (100 mM NaCl) or K^+ (100 mM KCl) or Mg^{2+} (10 mM $MgCl_2$) or Ca^{2+} (10 mM $CaCl_2$) or all these cations together, buffer named "IONS" (120 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$ and 2 mM $CaCl_2$)], or control buffer with choline chloride (100 mM). Tissues were incubated in triplicate (7315a and MtTW15 tumors) or in duplicate (EV-T and intact pituitary) for 75 min at 25° or for 30 min at 37°. At the end of the incubation, samples were diluted with 2 mL of cold washing buffer and filtered rapidly through Whatman (GF/C) glass fiber filters under reduced pressure using a cell harvester (Brandel). Following two 10-sec washes (4–6 mL each), the filters were placed into vials with 10 mL of scintillation fluid (Formula-963, from NEN Research Products, Boston, MA, U.S.A.). Radioactivity was monitored in a Beckman LS3801 instrument with an efficiency of 56%. Protein concentration was determined by the method of Lowry *et al.* [12].

Binding studies in 7315a and MtTW15 tumors were assayed in individual tumors, while pooled anterior pituitaries of 4–10 or 17–24 animals were used for saturation studies in EV-T and intact anterior pituitary respectively.

Statistical analysis. Scatchard plots were constructed from saturation curves, and least square linear regression analysis was performed to calculate the dissociation constant (K_D) and the maximum number of binding sites (B_{max}). For saturation experiments in 7315a, MtTW15 and EV-T, statistical evaluations of K_D and B_{max} values were performed by the nonparametric Wilcoxon signed-rank test (also

Table 1. Effects of cations and ionic strength on the dissociation constant (K_D) and density (B_{max}) of specific [^{3}H]spiperone binding to DA receptors in 7315a tumors*

Assay buffer	25° N	25° K_D (nM)	25° B_{max} (fmol/mg protein)	37° N	37° K_D (nM)	37° B_{max} (fmol/mg protein)
Control	12	0.056 ± 0.008	36 ± 6	10	0.067 ± 0.010	35 ± 4
Na^+	12	0.046 ± 0.008	31 ± 4	10	0.042 ± 0.006†	36 ± 5
Control	8	0.046 ± 0.008	43 ± 7	6	0.077 ± 0.015	32 ± 3
K^+	8	0.038 ± 0.007	35 ± 6	6	0.038 ± 0.003†	28 ± 4
Control	10	0.048 ± 0.007	45 ± 6	10	0.067 ± 0.010	35 ± 4
Mg^{2+}	10	0.061 ± 0.005	38 ± 6	10	0.054 ± 0.006	34 ± 6
Control	10	0.046 ± 0.006	45 ± 5	6	0.077 ± 0.015	32 ± 3
Ca^{2+}	10	0.057 ± 0.005‡	38 ± 6†	6	0.058 ± 0.003	31 ± 5
Control	6	0.038 ± 0.004	49 ± 5	9	0.066 ± 0.012	32 ± 3
IONS	6	0.034 ± 0.007	38 ± 3†	9	0.041 ± 0.002§	30 ± 3
Control	3	0.040 ± 0.005	42 ± 5	3	0.044 ± 0.010	32 ± 7
Choline	3	0.037 ± 0.008	33 ± 1	3	0.038 ± 0.011	32 ± 5

* The composition of the assay buffers was as follows: control buffer: 15 mM Tris–HCl, 100 μM EDTA, 12.5 μM nialamide, 0.01% ascorbic acid, pH 7.45, at 0–4°; control buffers with cations: Na^+ (100 mM NaCl) or K^+ (100 mM KCl) or Mg^{2+} (10 mM $MgCl_2$) or Ca^{2+} (10 mM $CaCl_2$) or all these cations together (IONS: 120 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$ and 2 mM $CaCl_2$); or control buffer with choline chloride (100 mM). DA receptors were assayed at 25° or 37° in 7315a tumors grown in intact female rats. Values are means ± SE obtained from N separate determinations.

† $P < 0.05$ vs control.

‡ $P = 0.0593$ vs control.

§ $P = 0.0663$ vs control.

called the Wilcoxon test for paired observations). K_D and B_{max} values obtained from an experiment using the same membrane preparation with the control buffer or the control buffer including a cation under study were paired for the test. For binding studies in intact anterior pituitary, statistical evaluations of K_D and B_{max} values were performed by an unpaired t-test (one-tailed or two-tailed).

RESULTS

Dopamine receptors were investigated in 7315a tumors which actively secrete, PRL plasma levels in rats bearing these tumors ranging from 1000 to 3000 ng/mL. Examples of [^{3}H]spiperone high-affinity binding to DA receptors in 7315a tumors and its modulation by cations are shown in Fig. 1. In 7315a tumors, modulation of DA receptor binding by cations was temperature dependent (Table 1). At 25°, the presence of Na^+, K^+, Mg^{2+}, Ca^{2+} or all these cations (IONS) did not affect the K_D of [^{3}H]spiperone binding compared to the K_D obtained using a buffer without ions. When the assays were performed at 37°, monovalent cations decreased the K_D values whereas they remained unchanged with divalent cations and IONS. The density (B_{max}) of [^{3}H]spiperone binding to DA receptors in 7315a tumors assessed at 25° was not changed by Na^+, K^+ and Mg^{2+}, whereas it was decreased in the presence of Ca^{2+} or IONS. At 37°, B_{max} was not affected by the cations under study. In 7315a tumors, at 25° and 37°, choline chloride did not change the K_D and B_{max} of [^{3}H]spiperone binding compared to values obtained using a buffer without ions, indicating that the effects of the cations are specific and not due to differences in ionic strength.

The MtTW15 tumors investigated actively secrete, PRL plasma levels in rats bearing these tumors ranging from 4500 to 35,000 ng/mL. Examples of [^{3}H]spiperone high-affinity binding to DA receptors in MtTW15 tumors and its modulation by cations are shown in Fig. 2. In MtTW15 tumors as for 7315a tumors, the ionic regulation of DA receptor antagonist binding site was temperature dependent (Table 2). At 25°, Na^+ did not change the K_D of [^{3}H]spiperone binding compared to the K_D obtained with a control buffer, whereas a decrease was observed at 37°. In contrast, K^+, Mg^{2+} and Ca^{2+} increased the K_D of [^{3}H]spiperone at 25°, while these cations had no effect on the K_D values at 37°. In the presence of IONS the K_D remained unchanged at both 25° and 37°. [^{3}H]Spiperone binding density at 25° in MtTW15 tumors was decreased by K^+ and unchanged by the other cations, whereas at 37° none of the cations studied affected it. At 25° or 37°, in MtTW15 tumors as for 7315a tumors, the K_D and B_{max} of [^{3}H]spiperone binding obtained in the presence of choline chloride was not different from values obtained with the control buffer, indicating the specificity of the ionic effects observed. These results show a different regulation of DA receptors by cations in 7315a and MtTW15 tumors.

After 30 weeks of estradiol valerate treatment, plasma PRL concentrations of treated animals

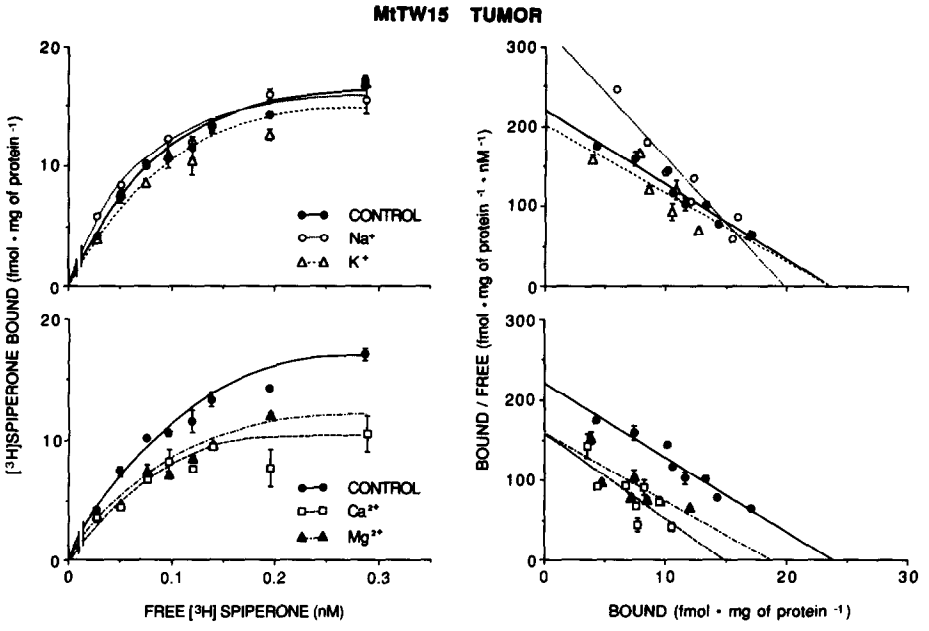

Fig. 2. Representative saturation experiments showing the effects of cations on [^{3}H]spiperone binding to DA receptors in MtTW15 tumors assayed at 37°. The concentrations of the cations were: 100 mM NaCl or 100 mM KCl as well as 10 mM $MgCl_2$ or 10 mM $CaCl_2$. The K_D values were 0.109 ± 0.012, 0.062 ± 0.007, 0.123 ± 0.028, 0.129 ± 0.037 and 0.102 ± 0.029 nM for the control, Na^+, K^+, Mg^{2+} and Ca^{2+} experiments respectively. The maximal binding capacity was respectively, 23 ± 4, 19 ± 4, 24 ± 8, 20 ± 8 and 16 ± 7 fmol/mg protein. Values are means ± SE.

Table 2. Effects of cations and ionic strength on the dissociation constant (K_D) and density (B_{max}) of specific [^{3}H]spiperone binding to DA receptors in MtTW15 tumors*

	25°			37°		
Assay buffer	N	K_D (nM)	B_{max} (fmol/mg protein)	N	K_D (nM)	B_{max} (fmol/mg protein)
Control	8	0.064 ± 0.008	29 ± 3	12	0.153 ± 0.024	29 ± 3
Na^+	8	0.078 ± 0.015	26 ± 3	12	0.110 ± 0.019†	24 ± 2‡
Control	6	0.058 ± 0.010	32 ± 4	11	0.160 ± 0.025	31 ± 2
K^+	6	0.090 ± 0.011†	25 ± 3†	11	0.184 ± 0.036	30 ± 3
Control	9	0.116 ± 0.032	56 ± 12	12	0.153 ± 0.024	29 ± 3
Mg^{2+}	9	0.238 ± 0.091§	48 ± 8	12	0.168 ± 0.031	30 ± 4
Control	6	0.058 ± 0.010	32 ± 4	12	0.153 ± 0.024	29 ± 3
Ca^{2+}	6	0.087 ± 0.006†	30 ± 3	12	0.172 ± 0.024	29 ± 4
Control	16	0.149 ± 0.024	79 ± 18	11	0.227 ± 0.031	41 ± 8
IONS	16	0.151 ± 0.027	59 ± 7	11	0.243 ± 0.040	34 ± 4
Control	13	0.205 ± 0.037	105 ± 23	6	0.234 ± 0.050	53 ± 12
Choline	13	0.267 ± 0.037	60 ± 8	6	0.282 ± 0.054	38 ± 6

* The composition of the assay buffers was as follows: control buffer: 15 mM Tris–HCl, 100 μM EDTA, 12.5 μM nialamide, 0.01% ascorbic acid, pH 7.45, at 0–4°; control buffers with cations: Na^+ (100 mM NaCl) or K^+ (100 mM KCl) or Mg^{2+} (10 mM $MgCl_2$) or Ca^{2+} (10 mM $CaCl_2$) or all these cations together (IONS: 120 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$ and 2 mM $CaCl_2$); or control buffer with choline chloride (100 mM). DA receptors were assayed at 25° or 37° in MtTW15 tumors grown in intact female rats. Values are means ± SE obtained from N separate determinations.

† $P < 0.05$ vs control.

‡ $P = 0.0597$ vs control.

§ $P < 0.01$ vs control.

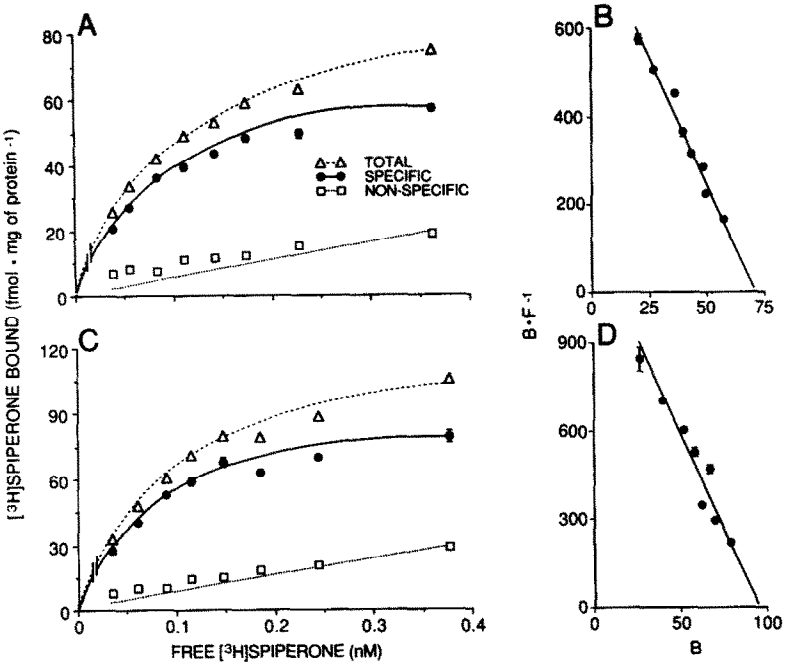

Fig. 3. Representative saturation experiments of [^{3}H]spiperone binding to DA receptors in intact anterior pituitary (A, B) and in EV-T (C, D) assayed at 37° in the presence of 100 mM NaCl. The K_D values for experiments in intact anterior pituitary and EV-T were, respectively, 0.087 ± 0.006 and 0.084 ± 0.009 nM. The maximal binding capacity was, respectively, 70 ± 7 and 98 ± 17 fmol/mg protein. Scatchard plots showing bound/free (B/F) data in fmol/mg protein/nM as a function of bound (B) ligand in fmol/mg protein are included in panels B and D. Values are means ± SE.

reached very high concentrations (3900 ± 200 ng/mL) compared to controls (60 ± 2 ng/mL; df = 163, P = 0.0001). Rat intact anterior pituitary and EV-T contained DA receptors of high affinity labeled with [^{3}H]spiperone (Fig. 3). At 37° in intact rat pituitary, Na^+ significantly decreased the K_D of [^{3}H]spiperone binding and left the B_{max} unchanged compared to values obtained using a control buffer (Table 3). In the presence of Ca^{2+}, no change in the affinity or density of [^{3}H]spiperone binding was observed. At 37°, in EV-T, the K_D of [^{3}H]spiperone binding was decreased by monovalent cations and Mg^{2+}, whereas it remained unchanged in the presence of Ca^{2+} (Table 3). [^{3}H]Spiperone binding density was unaffected by monovalent cations, whereas divalent cations increased it. In EV-T as for the other pituitary tumors studied, at 25° and 37° choline chloride did not change the K_D and B_{max} of [^{3}H]spiperone binding compared with values obtained with a control buffer, indicating the specificity of the ionic effects observed.

DISCUSSION

The 7315a, MtTW15 and EV-T tumors studied actively secrete PRL as measured by the high plasma levels of this hormone, in agreement with previous observations [4, 5, 8–11, 13, 14]. In addition, with [^{3}H]spiperone binding saturation experiments we confirmed the presence of a high-affinity DA antagonist binding site in these three tumor types; the affinity and density of [^{3}H]spiperone binding to DA receptors in these tissues are in agreement with those previously observed [4, 5, 7, 8, 10].

In the intact porcine adenohypophysis, Na^+ increases [^{3}H]spiperone binding affinity to D-2 DA receptors and leaves density unaffected [1–3]; this is observed independently of assay temperature, similar results being obtained in assays performed at 4°, 20° or 37° [2]. For comparison we have used the same ionic concentrations and similar experimental conditions as George *et al.* [1] and Watanabe *et al.* [2, 3] for D-2 DA receptor binding studies to intact porcine adenohypophysis. Under these conditions, our results obtained in intact rat pituitary show that, as in intact porcine pituitary [1–3], Na^+ increases [^{3}H]spiperone binding affinity and leaves density unchanged. Na^+ is essential for a high-affinity interaction of sulpiride and other benzamides with DA receptors in human, rat and dog pituitary gland [15, 16]. This specific sodium dependence of benzamides is probably due to a reversible modification of the receptor membrane by this ion [16]. Moreover, a common feature of receptors which are negatively coupled to adenylate cyclase is their regulation by Na^+ [2]. In 7315a and MtTW15 tumors, modulation of DA receptors by Na^+ depends on assay temperature. Indeed, in these tumors assayed at 25°, Na^+ did not change [^{3}H]spiperone binding affinity and density in contrast to intact tissue, whereas at 37° Na^+ increased affinity and left density unaffected as for intact tissue. Thus, an abnormal modulation by Na^+ was seen when binding experiments were done

Table 3. Effects of cations and ionic strength on the dissociation constant (K_D) and density (B_{max}) of specific [^{3}H]spiperone binding to DA receptors in intact pituitary and estradiol-induced pituitary tumors (EV-T)*

Pituitary tissue	Assay buffer	N	K_D (nM)	B_{max} (fmol/mg protein)
Intact	Control	3	0.113 ± 0.008	68 ± 1
	Na^+	3	0.088 ± 0.002†	71 ± 2
	Control	4	0.091 ± 0.012	81 ± 10
	Ca^{2+}	4	0.080 ± 0.008	98 ± 12
EV-T	Control	8	0.105 ± 0.008	88 ± 7
	Na^+	8	0.075 ± 0.005†	87 ± 5
	K^+	8	0.078 ± 0.004†	84 ± 6
	Mg^{2+}	8	0.067 ± 0.002†	98 ± 7†
	Ca^{2+}	8	0.083 ± 0.005	100 ± 7†
	Choline	8	0.083 ± 0.007	82 ± 5

* The composition of the assay buffers was as follows: control buffer: 15 mM Tris–HCl, 100 μM EDTA, 12.5 μM nialamide, 0.01% ascorbic acid, pH 7.45, at 0–4°; control buffers with cations: Na^+ (100 mM NaCl) or K^+ (100 mM KCl) or Mg^{2+} (10 mM $MgCl_2$) or Ca^{2+} (10 mM $CaCl_2$) or control buffer with choline chloride (100 mM). DA receptors were assayed at 37° in intact pituitary and estradiol-induced pituitary tumors (EV-T) grown in intact female rats. Values are means ± SE obtained from N separate determinations.

† $P < 0.05$ vs control.

at 25°. In our previous experiments performed at 25° in 7315a tumors, the effect of the addition of Na^+ (corresponding to buffer "IONS" in the present studies, i.e. 120 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$ and 2 mM $CaCl_2$) was compared with the effect of a buffer containing all the other cations except sodium. Under these conditions, Na^+ increased DA receptor affinity [7]. This is at variance with the present results and is probably due to differences in assay buffers. In EV-T, Na^+ such as for intact tissue increased the affinity of the DA receptors and left density unchanged.

In the intact porcine anterior pituitary, K^+ increases [^{3}H]spiperone binding affinity [1]. DA was shown recently to increase K^+ permeability. This response of the K^+ flux to DA may lead to inhibition of PRL release by inactivation of the membrane calcium channels, which in turn leads to a decrease of cytosolic calcium [17]. In 7315a and MtTW15 tumors, modulation of DA receptors by K^+ depended on assay temperature. In 7315a tumors assayed at 25°, K^+ did not change [^{3}H]spiperone binding affinity and density, whereas at 37° it increased affinity and left density unchanged. In MtTW15 tumors assayed at 25°, K^+ decreased [^{3}H]spiperone binding affinity and density and at 37° left them unaffected. Thus, modulation by K^+ is the same as for intact tissue only in 7315a tumors assayed at 37°. In EV-T as in intact tissue, K^+ increased the affinity of the DA receptors and left density unchanged.

In the intact porcine adenohypophysis, monovalent cations (Na^+, K^+, or Li^+) similarly increase [^{3}H]spiperone binding affinity [1]. In 7315a tumors and EV-T, the effect of Na^+ was similar to the influence of K^+ on [^{3}H]spiperone binding affinity; however, in 7315a tumors the normal effect of these monovalent cations to increase affinity was observed only in the assays at 37°. By contrast, in MtTW15 tumors the effect of Na^+ on [^{3}H]spiperone binding affinity was different from the effect of the other monovalent cation, K^+, at both assay temperatures (25° and 37°).

In the intact porcine anterior pituitary, Mg^{2+} decreases the affinity of [^{3}H]spiperone binding whereas the density is unaffected [3]. In 7315a tumors, Mg^{2+} did not change [^{3}H]spiperone affinity and density when assayed at 25° or 37°. In MtTW15 tumors, modulation of DA receptors by Mg^{2+} depended on assay temperature. In these tumors assayed at 25°, Mg^{2+} decreased the affinity and left density unaffected, whereas at 37° Mg^{2+} left the DA receptors unchanged. In EV-T, Mg^{2+} increased the affinity and density. Thus, modulation by Mg^{2+} was normal only in MtTW15 tumors assayed at 25°.

In the intact porcine adenohypophysis, the effect of Mg^{2+} on [^{3}H]spiperone binding affinity is opposite to the effect of Na^+, i.e. Mg^{2+} decreasing and Na^+ increasing affinity [3]. In 7315a, MtTW15 and EV-T tumors this opposite effect of Na^+ compared to Mg^{2+} was not observed.

DA was shown recently to inhibit calcium influx, thereby reducing intracellular calcium levels, which leads to suppression of PRL release [18–20]. The mechanisms by which DA blocks calcium influx may be multiple, involving direct inhibition of a calcium channel associated with the D-2 DA receptor and/or indirect effects mediated through adenylate cyclase, phospholipid metabolism, or the plasma membrane K^+ channel [18] by an increase of the K^+ permeability of the cell [17]. While DA receptors are implicated in the regulation of calcium influx, the opposite was not observed in our study. Indeed, our experiments with Ca^{2+} in intact rat pituitary showed that this cation had no effect on affinity and density of [^{3}H]spiperone binding at 37°. In 7315a and MtTW15

tumors, modulation of DA receptors by Ca^{2+} depended on assay temperature. In 7315a tumors at 25°, Ca^{2+} had a tendency to decrease the affinity ($P = 0.0593$) and decreased significantly the density ($P < 0.05$); at 37° the affinity and density were not affected by Ca^{2+}. Modulation by Ca^{2+} at 25° was also abnormal in MtTW15 tumors, where Ca^{2+} decreased [^{3}H]spiperone binding affinity and left density unaffected, while at 37°, Ca^{2+} left the DA receptors unchanged as in the intact tissue. Thus, an abnormal regulation by Ca^{2+} of the DA receptors at 25° was observed in 7315a and MtTW15 tumors. It has been suggested that the refractoriness of 7315a tumor cells to DA agonist inhibition of basal PRL release may be due to the abnormal calcium balance of the tumor under basal conditions, DA inhibiting PRL release only when Ca^{2+} mobilization is increased [6, 21, 22]. However, in contrast to 7315a tumors, in MtTW15 tumors DA does not inhibit PRL release when Ca^{2+} mobilization is increased [23]. In EV-T, the affinity was unchanged and the density was increased by Ca^{2+}, also indicating an abnormal modulation by Ca^{2+}.

We have shown in 7315a and MtTW15 tumors that the modulation of DA receptors by monovalent and divalent cations depends on assay temperature. In 7315a and MtTW15 tumors, an abnormal modulation by Na^+ and Ca^{2+} was seen when binding experiments were done at 25°, whereas modulation by these ions became normal at 37°. The abnormal modulation of the DA receptors by K^+ in 7315a tumors at 25° was also normalized at the higher assay temperature. Inversely, in MtTW15 tumors a normal modulation by Mg^{2+} was observed at 25°, whereas modulation became abnormal at 37°. Moreover, the abnormal modulation by K^+ in MtTW15 tumors or by Mg^{2+} in 7315a tumors was seen at both assay temperatures, 25° and 37°. Therefore, our results suggest that the abnormal ionic modulations of the DA receptors observed do not depend only on assay conditions but reflect intrinsic properties of these receptors. In addition, normalization of the ionic effect at higher temperature in some cases may suggest thermodynamic factors implicated in these ionic effects; these factors may also be changed in tumoral tissues.

In summary, our results have shown that the regulation of the DA receptor antagonist binding site by monovalent and divalent cations is different in 7315a compared to MtTW15 tumors and also compared to intact tissue. This supports the suggestion by Lafond *et al.* [23] of the existence of a difference between 7315a and MtTW15 mechanisms of resistance to the DA inhibitory action and their location at two different sites. EV-T have high-affinity DA receptors modulated by the monovalent cations Na^+ and K^+ such as those receptors in the intact pituitary; however, their modulation by the divalent cations Mg^{2+} and Ca^{2+} is abnormal. The defective modulation by cations of DA receptors in these tumors may be implicated in the abnormal signal transduction from these receptors to inhibit PRL secretion.

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