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Desensitization of the dopaminergic system in bovine retina following incubation with high potassium

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The effect of potassium depolarization on dopamine D_1 receptor activity in bovine retina was investigated. Preincubation of bovine retinas in buffer containing high KCl (56 mM) as compared to a low KCl control buffer resulted in a significant decrease in dopamine-stimulated adenylate cyclase activity with no change in basal or GTP-stimulated adenylate cyclase activity. The apparent V_{max} for dopamine was decreased from 102 ± 15 pmol/min/mg protein in retinas preincubated in high KCl to 71 ± 11 pmol/min/mg protein in control retinas ($n = 5$). The apparent K_a for dopamine stimulation of the enzyme did not change. The potassium-induced desensitization could be blocked by preincubation with the dopamine antagonist *cis*-flupenthixol suggesting that the desensitization was caused by the release of dopamine. The rapid desensitization was not accompanied by a change in D_1 receptor density as assessed by binding of [^{3}H]SCH23390 nor in agonist binding as assessed by competition of the selective D_1 agonist, SKF38393, for [^{3}H]SCH23390 binding. The potassium-induced desensitization was mimicked by preincubation of retinas in control medium containing isobutylmethylxanthine or dibutyryl cyclic AMP. Incubation of retinas in 56 mM KCl also led to a decrease in activation of adenylate cyclase by vasoactive intestinal polypeptide. These results strongly suggest that potassium depolarization leads to a very rapid heterologous desensitization of adenylate cyclase in bovine retinas.

INTRODUCTION

Dopamine (DA) has been well established as a neurotransmitter in the retina and this tissue has been extensively used to investigate the DA neurotransmitter system. DA-containing neurons are located among the amacrine cells in the inner nuclear layer of the retina[7]. DA D_1 receptors present in the inner retina stimulate adenylate cyclase activity[4,26]. Electrophysiological studies have shown that DA affects the receptive field properties of ganglion cells in mammalian retina[1,30,32]. In rabbit retina, D_1 receptor antagonists were found to affect the spontaneous activity of on- and off-center ganglion cells and to abolish the antagonist surround[11,12]. D_1 receptors have also been shown to mediate acetylcholine release in mammalian retina[9]. In fish retinas, D_1 receptor activation has been shown to uncouple electrical synapses between horizontal cells[31].

DA has been shown to be released from its neurons in retina in response to light[2,15]. DA released in response to light could act at postsynaptic D_1 receptors and subsequently alter the responsiveness of that receptor. In previous studies, we found a rapidly-occurring desensitization of DA-stimulated adenylate cyclase activity in membrane fractions of bovine retina that occurred after preincubation of the retinas under light as compared to dark conditions[6]. The desensitization was manifested as a significant decrease in the apparent V_{max} for DA, with no change in the apparent K_a. Preincubation of the retinas with DA produced a desensitization of the DA-stimulated adenylate cyclase activity that resembled that of light, supporting the conclusion that release of DA mediated the light-induced desensitization of the enzyme activity.

One caveat to those experiments is that rhodopsin could not be continuously regenerated in the isolated bovine retina under the preincubation conditions. In order to further investigate the molecular mechanism of the desensitization of the D_1 receptor in bovine retina, a depolarizing concentration of potassium was used to release DA. Incubation of retinas with depolarizing concentrations of potassium has been shown to release DA and increase cAMP content in mammalian, amphibian and fish retinas[2,5,25]. The increase in cAMP can be blocked by DA receptor antagonists[5,28], further demonstrating that K^+ depolarization releases DA.

In this report, we demonstrate a rapid desensitization of DA-sensitive adenylate cyclase in mammalian retinas in response to potassium-induced depolarization. We then examine whether the desensitization is due to a

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change at the level of the receptor or an alteration in the coupling of the receptor to adenylate cyclase. Our studies indicate that potassium depolarization leads to a desensitization of adenylate cyclase activity that is not specific for DA. The results are compatible with development of heterologous desensitization which is due to an uncoupling of receptors from the stimulatory GTP-binding protein, Gs[8,27].

MATERIALS AND METHODS

Tissue treatment

Bovine eyes were obtained from a slaughterhouse shortly after the animals' death, maintained in the dark and transported on ice. Retinas were dissected from the eyes in a dark room under a dim red light (Kodak Adjustable Safelight Lamp Model B). The eye was hemisected, and the back of the eyeball was inverted, releasing the vitreous humor. The retina was gently removed from the underlying choroid and sclera using forceps and placed in physiological buffer.

Treatment with high potassium

The physiological buffer used contained 30 mM HEPES buffer, pH 7.4, 1.7 mM $CaCl_2$, 2.9 mM $NaHCO_3$, 1.0 mM $MgCl_2$, 1 mg/ml glucose, KCl and NaCl. The buffer for high potassium treatment contained 56 mM KCl and 91.6 mM NaCl, while buffer for control retinas contained 5.6 mM KCl and 142 mM NaCl. Incubations were conducted for 15 min at 37 °C in the dark room immediately after dissection of retinas. Retinas were placed in previously oxygenated control or treatment buffer in airtight plastic tubes in a ratio of 0.75 ml buffer per retina. The reaction was stopped by diluting the tissue with 4 vols. of the buffer used for adenylate cyclase or binding assays. The tissue was centrifuged and washed 3 times with the appropriate buffer. Treatment groups were paired within a given experiment to minimize errors associated with interassay variability.

Adenylate cyclase assays

Membrane fractions were prepared by homogenizing the tissue in 10 vols. of 20 mM HEPES buffer, pH 7.5, containing 1.2 mM EGTA and 5 mM $MgSO_4$ in a glass teflon homogenizer. The homogenate was centrifuged at 27,000 *g* for 20 min and washed twice. The resulting pellet was resuspended in the HEPES buffer. The assay, in a volume of 200 μl, contained 20 mM HEPES, pH 7.5, 5 mM $MgCl_2$, 2 mM cAMP, 4 mM phosph*enol*pyruvate (PEP), 20 μg pyruvate kinase, 0.12 mM isobutylmethylxanthine (IBMX), 0.5 mM [α-^{32}P]ATP (1 μCi/assay), and 25 μl of particulate membrane fraction to give a protein concentration of 0.1 mg/ml, with or without the addition of 1 μM GTP, DA or vasoactive intestinal polypeptide (VIP). DA- or VIP-stimulated adenylate cyclase activities were calculated as the pmol of cAMP/min/mg protein produced above that activity in the presence of GTP alone. Assays were incubated for 8 min at 37 °C and the reaction was stopped by heating for 1 min at 95 °C, followed by addition of 200 μl of a solution containing 20 mM ATP and 0.7 mM [^{3}H]cAMP. The particulate material was centrifuged and the ^{32}P-labeled cAMP in the supernatant fraction was separated from [^{32}P]ATP over a Biorad AG-50 resin column followed by precipitation with $Ba(OH)_2$ and $ZnSO_4$ according to Krishna et al.[16]. Recovery of cAMP was measured using the [^{3}H]cAMP and was usually 80–90%.

[^{3}H]SCH 23390 binding assay

Membrane fractions were prepared as described for adenylate cyclase assays with two exceptions. Retinas were homogenized in 20 mM HEPES buffer, pH 7.8, containing 1.2 mM EGTA, 5 mM $MgSO_4$ and 0.32 M sucrose with a Brinkman polytron for 10 s at setting 6 prior to centrifugation. The assay, in a volume of 1 ml, contained [^{3}H]SCH 23390, unlabeled competing drug or buffer, 0.1% tartaric acid and 800 μl of membranes to give a protein concentration of 0.1 mg/ml. Samples were incubated for 90 min at 25 °C. Non-specific binding was determined in the presence of 10 μM *cis*-flupenthixol. The binding reaction was terminated by rapid filtration over Whatman GF/C filters and immediate rinsing 4 times with 4 ml of ice-cold 50 mM Tris HCl buffer, pH 7.7. The filters were allowed to air dry for 30 min, placed in xylene-based Aqueous Counting Scintillant (ACS) and shaken for 30 min. Radioactivity was determined by liquid scintillation spectrophotometry.

Protein measurement

Protein was determined by the method of Lowry et al.[17].

Data analysis

Statistical analysis was performed using paired Student *t*-test. K_d and B_{max} values for [^{3}H]SCH23390 binding were determined from Scatchard analysis. The data fit to a single site and linear regression was used to calculate the actual values. A possible difference in K_d or B_{max} values for [^{3}H]SCH23390 binding for control and KCl-treated retinas was also assessed by non-linear regression analysis using Graph PAD (ISI, Philadelphia, PA). The data were first analyzed simultaneously with the K_d and B_{max} values both constrained to be estimated as a common value between treatment groups (pooled fit). The data were then analyzed such that both parameters could vary between control and KCl treatment groups (separate fit). The improvement in fit produced by relaxing the constraints was assessed using the variance ratio (*F* statistic) derived from the residual variance (the ratio of the sum of squares of the residuals divided by the degrees of freedom) as described by Motulsky and Ransnas[19]. The *F* ratio was calculated using the equation $F = [(SS_{pool} - SS_{sep})/df_{pool} - df_{sep})]/(SS_{sep}/df_{sep})$ where SS_{pool} = sum of squares of the combined fit, SS_{sep} = sum of squares of the separate fits, df_{pool} = the degrees of freedom of the combined fit and df_{sep} = degrees of freedom of the separate fit. The curves for competition of SKF38393 for [^{3}H]SCH23390 binding were fit to a two site model using Graph PAD. The curves were analyzed with the K_i values for the two sites estimated to be a common value and with the constraints relaxed. The improvement in fit produced by relaxing the constraints was assessed as described above.

Materials

[α-^{32}P]ATP (30 Ci/mmol) and [^{3}H]SCH 23390 (76 Ci/mmol) were obtained from Amersham Searle, Arlington Heights, IL. ATP, cAMP, PEP and BSA were purchased from Sigma Chemical Co., St. Louis, MO. GTP and pyruvate kinase were from Boehringer-Mannheim, Indianapolis, IN. *Cis*-flupenthixol was graciously donated by H. Lunbeck, A/S, Copenhagen, Denmark. SKF38393 was generously donated by Smith Kline and French Pharmaceuticals, Philadelphia, PA.

RESULTS

Following incubation with 56 mM KCl, there was a significant decrease in stimulation of adenylate cyclase by DA in retinal membranes as compared to membranes from retinas preincubated in low potassium (Fig. 1). Kinetic analysis of enzyme activity using a Hanes plot demonstrated that there was a significant difference in the apparent V_{max} for DA in retinas treated with high KCl, as compared to the low KCl control. The apparent V_{max} of the reaction decreased from 102 ± 15 pmol/min/mg protein for control retinas to 71 ± 11 pmol/min/mg protein for high KCl-treated retinas ($n = 5$, $P < 0.05$, two-tailed paired *t*). The apparent K_a value of the enzyme was not significantly different between the two groups, being 1.5 (1.2–1.8) and 1.7 (1.4–2.0) μM for

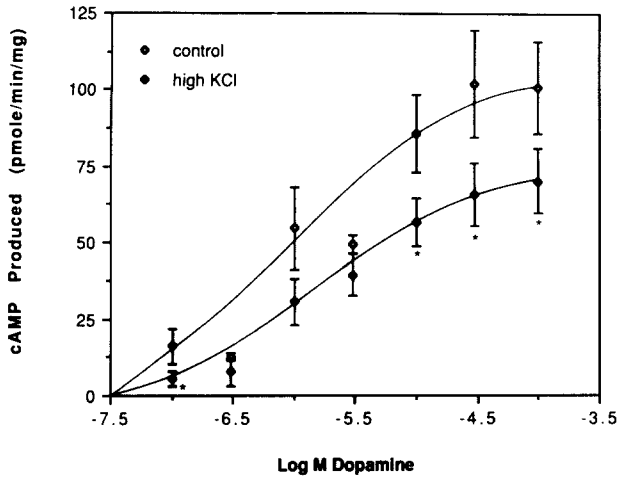

Fig. 1. DA-stimulated adenylate cyclase activity in bovine retina after preincubation with high KCl or under control conditions. Activation of adenylate cyclase by DA was measured in membranes prepared from retinas incubated in physiological buffer containing low or with high KCl as described in Methods. DA-stimulated activity was calculated as described in Methods. Adenylate cyclase activities in the presence of GTP are given in the text. Each point represents the average ± SE of 5 separate determinations performed in triplicate. $^*P < 0.05$ for treatment as compared to control, paired *t*-test.

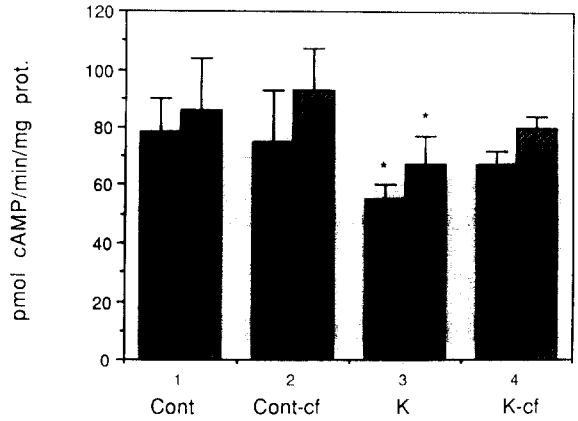

Fig. 2. Effect of 500 nM *cis*-flupenthixol on potassium-induced decrease in DA-stimulated adenylate cyclase activity in bovine retina. Retinas were preincubated under control (cont) conditions or in the presence of 56 mM KCl (K) in the absence or presence (cont-cf, K-cf) of 500 nM *cis*-flupenthixol as described in Methods. The results are shown as the DA-stimulated adenylate cyclase activity for 10 μM (solid bars) and 100 μM (striped bars) DA in pmol of cAMP/min/mg protein. Results are the average of 4 experiments ± S.E. each performed in triplicate. Adenylate cyclase activities in the presence of 1 μM GTP in pmol/min/mg prot. for cont, cont-cf, K and K-cf groups were: 61 ± 6, 57 ± 8, 55 ± 8 and 39 ± 9, respectively. $^*P < 0.05$ as compared to cont and K-cf conditions, paired *t*-test. $^{**}P < 0.05$ as compared to cont, cont-cf, and K-cf conditions, paired *t*-test.

control and high KCl-treated retinas, respectively. The apparent K_a values were averaged as the logarithm base 10 therefore the standard error (numbers in parentheses) is expressed as a range.

The basal adenylate cyclase activities in the control and treatment groups were not significantly different, being 59 ± 11 vs 47 ± 4 pmol/min/mg protein, respectively (n = 5). Activities measured in the presence of 1 μM GTP in the control and treatment groups were also not significantly different (67 ± 11 vs 49 ± 4 pmol/min/mg protein, respectively (n = 5). The control values appear somewhat higher than treatment values due to one high control value that skewed the average.

To further determine whether the desensitization produced by depolarization was due to a release of DA, the ability of a DA receptor antagonist to block the desensitization was examined. Retinas were preincubated under low (control) and high KCl conditions with and without 500 nM *cis*-flupenthixol. The inclusion of 500 nM *cis*-flupenthixol in the preincubation blocked the KCl-induced decrease in stimulation of adenylate cyclase by 10 and 100 μM DA as shown in Fig 2. *Cis*-flupenthixol

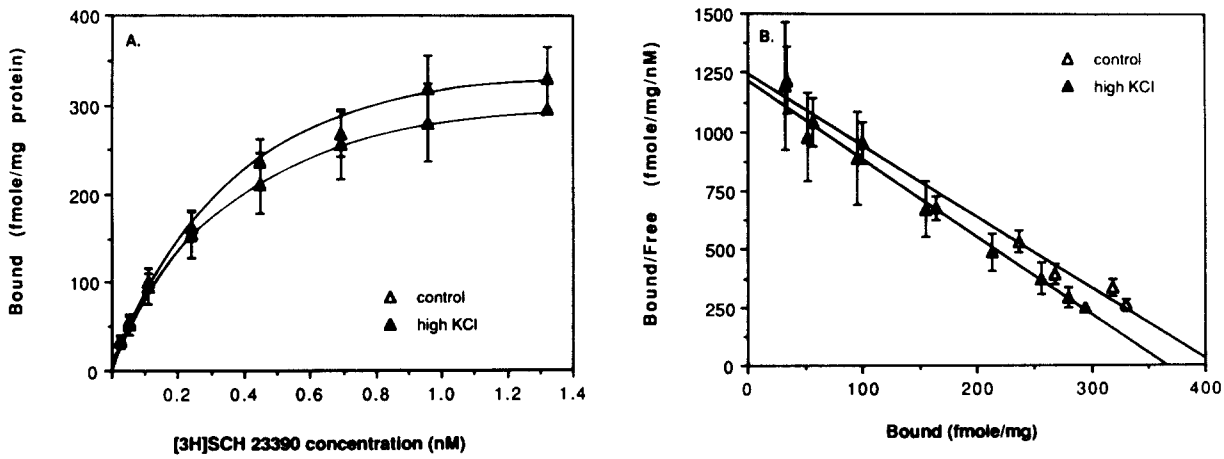

Fig. 3. Binding of [^{3}H]SCH23390 to bovine retinal membranes after preincubation under control conditions or with high KCl. Binding of [^{3}H]SCH23390 to retinal membranes was measured as described in Methods. A: saturation curve for [^{3}H]SCH23390 binding. B: Scatchard analysis of data in A. Each point represents the mean ± S.E. for 5 separate experiments. Kinetic constants are given in text.

itself had no effect on DA activation under control conditions.

The possibility that the desensitization was due to a change in number or affinity of D_1 receptors was examined by measuring the binding of a labeled antagonist to D_1 receptors on the membranes after preincubation in high KCl or control conditions. The level of D_1 receptors in retinas was measured initially with the specific D_1 antagonist, [^{3}H]SCH23390. Saturation curves representing specific binding of [^{3}H]SCH23390 to retinal membranes after preincubation in high KCl or control conditions are shown in Fig. 3. The data in Fig. 3A demonstrate that there was no significant difference in the affinity or density of D_1 receptors present in retinas preincubated under control conditions or with high KCl. Scatchard analysis (Fig. 3B) of the saturation experiments produced B_{max} values of 415 ± 38 fmol/mg protein and 371 ± 43 fmol/mg protein for control and high KCl treatment groups, respectively (n = 5). The K_d values for [^{3}H]SCH23390 were 0.32 (0.298–0.35) nM and 0.33 (0.27–0.40) nM for control and treatment groups, respectively. The K_d values were averaged as the logarithm base 10, therefore the standard errors are given as a range. The results were also analyzed using non-linear regression analysis with the program Graph PAD. The combined saturation curves from 5 control and 5 KCl-treated retinas were fit simultaneously with no constraints placed on the analyses. The average B_{max} values for control and treatment retinas calculated by this analysis were, in fmol/mg protein, 435 ± 35 and 387 ± 47, respectively. It was found that the fit by non-linear least square analysis fitted with combined and separate F-test showed no improvement with sum of squares residuals (F = 1.09, $P > 0.05$). Therefore neither the B_{max} nor K_d values for the two groups were significantly different.

Although no difference in receptor density was detected by measuring antagonist binding, it is possible that there could be a selective alteration of D_1 agonist binding

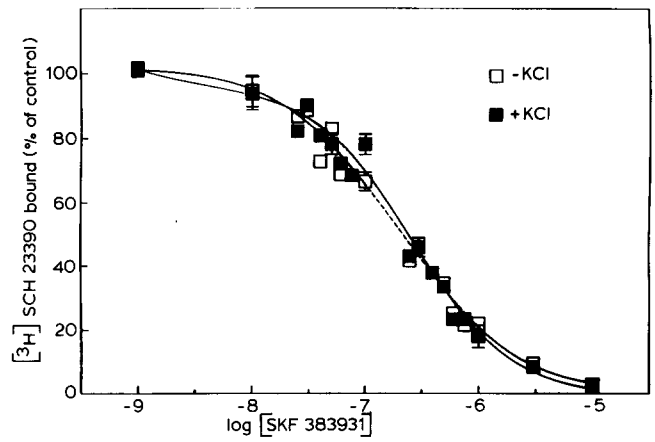

Fig. 4. Competition for [^{3}H]SCH23390 binding sites by SKF38393 following preincubation of bovine retinas with low or high KCl. Non-specific binding was determined with 10 μM *cis*-flupenthixol. The concentration of [^{3}H]SCH23390 was 0.15 nM. Each point represents the average of one to 3 separate experiments conducted in triplicate.

sites, which would be detected by measuring agonist competition with labeled [^{3}H]SCH23390. Competition for [^{3}H]SCH 23390 binding by the D_1 receptor agonist SKF38393 was measured following treatment with high KCl (Fig. 4). The curves were fit to a two site analysis using Graph PAD. In 3 separate experiments, no difference was found between control and treatment groups in the ability of the selective D_1 agonist SKF38393 to compete for [^{3}H]SCH23390 binding sites. The K_i values for SKF38393 competition for the combined control experiments were 43 nM and 280 nM for the high and low affinity sites, respectively, and 40% of the sites were of high affinity.The K_i values for SKF38393 competition for the combined KCl treatment experiments were 2 nM and 180 nM for the high and low affinity sites, respectively, and 19% of the sites were of high affinity. Calculation of the variance ratio (F statistic) as described by Motulsky and Ransnas[19] demonstrated that there was

TABLE I

Effect of preincubation with IBMX or dbcAMP on activation of adenylate cyclase by 100 μM DA in bovine retina

Adenylate cyclase activity is expressed as pmol of cAMP/min/mg protein produced by 100 μM DA above that activity in the presence of 1 μM GTP. Adenylate cyclase activities in the presence of GTP, in pmol/min/mg protein fopr Expt. 1 control, KCl, IBMX treatment and IBMX + KCl are: 31 ± 2, 34 ± 5, 48 ± 8 and 31 ± 5, respectively. Adenylate cyclase activities in the presence of GTP, in pmol/min/mg protein, for Expt. 2 are: 45 ± 5, 37 ± 6 and 45 ± 5, respectively. n.d. = not determined.

	DA-stimulated adenylate cyclase activity (pmol/min/mg prot.)				
	n	*Control*	*56 mM KCl*	*Treatment*	*Treatment + KCl*
Expt. 1: 2 mM IBMX	4	68 ± 8	28 ± 8*	32 ± 4*	41 ± 7**
Expt. 2: 1 mM dbcAMP	3	94 ± 3	75 ± 5**	80 ± 3*	n.d.

*$P \leq 0.05$ with respect to corresponding control, paired t-test.
**$P \leq 0.05$ with respect to Expt. 2 control, unpaired t-test.

TABLE II

Stimulation of adenylate cyclase activity by VIP following incubation of bovine retinas with 56 mM KCl

VIP-stimulated adenylate cyclase activity is expressed as the pmol of cAMP produced/min/mg protein above that activity in the presence of 1 μM GTP. Adenylate cyclase activities in the presence of 1 μM GTP for control and 56 mM KCl preincubations are: 55 and 42 pmol/min/mg protein, respectively. Results are the average of 2 experiments, performed in triplicate, that differed less than 10%.

VIP concentration (mM)	*VIP-stimulated adenylate cyclase activity (pmol/min/mg prot.)*	
	Control	*56 mM KCl*
1	29	15
10	34	24
100	75	31

no improvement in fit produced by relaxing the constraints between the control and treatment values. Therefore there was no significant difference in agonist binding to [^{3}H] SCH23390 binding sites between the control and KCl treatment groups ($F = 0.68$, $p \geq 0.05$).

The lack of change in D_1 receptor binding suggested that potassium depolarization could have produced a heterologous desensitization which is partially mediated by cAMP[27]. To examine this possibility, retinas were preincubated with IBMX, a phosphodiesterase inhibitor, and dibutyryl cAMP. As shown in Table I, there was a significant decrease in pmol of cAMP produced/min/mg protein in retinas preincubated under control conditions with 2 mM IBMX as compared to retinas preincubated solely under control conditions. Preincubation of retinas with KCl+IBMX produced no greater desensitization than that induced by either agent alone. Similarly, preincubation of retinas in the presence of 1 mM dibutyryl cAMP also produced a desensitization of DA-stimulated adenylate cyclase activity. Neither IBMX nor dibutyryl cAMP altered the basal or GTP-stimulated adenylate cyclase activity in any group.

We then determined whether the desensitization produced by potassium depolarization was specific for the D_1 DA receptor. Following incubation of retinas with control or high potassium buffer, the stimulation of adenylate cyclase activity by VIP was measured. As shown in Table II, the stimulation of adenylate cyclase activity was decreased in retinas preincubated with high KCl at each concentration of VIP measured.

DISCUSSION

We have found that potassium depolarization elicited a rapid desensitization of D_1 receptor stimulation of adenylate cyclase activity in bovine retinas. It is likely that the DA receptor desensitization was mediated by a release of DA from retinal neurons. It has been shown that incubation of retinas, including calf retina, with potassium increases cAMP content in the retina and this can be blocked with DA receptor antagonists[5,28]. In this study it was found that the potassium-induced desensitization of the DA-stimulated adenylate cyclase activity could be blocked with *cis*-flupenthixol, a DA receptor antagonist.

The desensitization was manifested by a decrease in apparent V_{max} for DA-stimulated adenylate cyclase activity with no change in affinity for DA. There was no measurable change in DA receptor density or agonist affinity as determined by binding of the antagonist [^{3}H]SCH23390 or by agonist competition for antagonist binding. The simplest explanation for the results is that potassium releases DA, which activates postsynaptic D_1 receptor and brings about a desensitization of that receptor. Another possible explanation for the results is that KCl would induce an increase in D_2 receptor activity, which inhibits adenylate cyclase[29], causing a resultant decrease in D_1-mediated stimulation. This would require that D_1 and D_2 receptors be located on the same postsynaptic membrane and be functionally coupled. Using autoradiography, Brann and Young[3] found that D_2 receptor-specific ligands densely labeled rods in bovine retina but there was very little specific labeling in the inner nuclear or inner plexiform layers. D_2 receptors that are present in the inner layers of the bovine retina have been shown to modulate release of DA from cells[25]. Pachter and Lam[21] found that neither D_2 agonists nor antagonists altered DA-stimulated adenylate cyclase activity in rabbit retinal homogenates. Qu et al.[24], however, recently reported that inhibition of adenylate cyclase by DA and D_2 agonists could be measured in intact rat retina after preincubation with SCH23390. These results suggest that D_1 and D_2 receptors could be functionally coupled on the rat retinal membrane. If this is true in bovine retina, the KCl-mediated release of DA, while initially activating D_2 receptors, would probably result in a desensitization of D_2 receptors thereby decreasing the D_2 activity upon subsequent measurement in an assay. Further, preliminary experiments using SKF38393, a selective D_1 agonist, instead of DA also demonstrated the desensitization. The experiments demonstrating the blockade of the KCl-induced desensitization with *cis*-flupenthixol do not answer this question since *cis*-flupenthixol has nearly equal affinity for D_1 and D_2 receptors. The studies of VIP activation and IBMX- and dbcAMP-induced desensitization coupled with the lack of change of D_1 receptor binding, however, strongly suggest that the desensitization induced by potassium is heterologous in nature. In heterologous desensitization, no change is found in receptor binding characteristics, but

there is a decrease in activation of adenylate cyclase by the desensitizing hormone/neurotransmitter and other neurotransmitters that are coupled to the common adenylate cyclase catalytic subunit[8,27]. There may be a slight decrease in activity of agents that act at Gs such as GTP. In most of our experiments, stimulation by GTP was unaltered by KCl treatment, although in some assays (see legend to Fig. 2) there were decreases in GTP stimulation. In this study, we found a decrease in stimulation of adenylate cyclase by both DA and VIP in retinas after preincubation in high potassium. There is some evidence that DA and VIP receptors can exist on the same cells in retina. Pachter and Lam[21] have found that both DA and VIP stimulate adenylate cyclase in homogenates of rabbit retina in a non-additive manner. VIP activation of adenylate cyclase has also been demonstrated in isolated horizontal cells of fish retina that contain DA receptors[33]. The potassium-induced desensitization could be mimicked by IBMX and dibutyryl cAMP suggesting that a cAMP-dependent phosphorylation partially mediates the desensitization as has been demonstrated for heterologous desensitization[27] of the β-adrenergic receptor.

The preincubation of retinas with high concentrations of potassium could mimic the effect of light on the retinas. Light has been shown to release DA from its neurons in retinas[2,15]. It has been demonstrated that the light-stimulated release of DA in rabbit retina is similar to that evoked by membrane depolarization by high potassium concentrations or electric current[2,20]. Light has also been shown to regulate DA D_1 receptor activity in mammalian retinas. We have previously shown that preincubation of bovine retinas for 20 min under light conditions produced a desensitization of DA-stimulated adenylate cyclase as compared to those preincubated under dark conditions[6]. The light-induced desensitization could be mimicked by preincubation with DA. Porceddu et al.[22] found a greater activation of adenylate cyclase activity by DA in retinas from rats subjected to 4 h of dark adaptation as compared to light-adapted animals. In contrast to our results, they found an increase in the density of [^{3}H]SCH23390 binding in the retinas of dark-adapted rats which corresponded with the increase in DA stimulation of the enzyme. Our studies of desensitization induced by light and potassium involved very short-term treatments with these agents. Therefore, a rapid desensitization of DA receptors in the retina may not involve changes in receptor density or binding characteristics while changes in these parameters do occur after longer-term treatments.

Electrophysiological studies in retina suggest that one function of the dopaminergic system in retina may be to modulate the response of ganglion cells to light. It has been shown that DA affects the electrophysiological properties of ganglion cells in cat and rabbit retina[1,10–12,18,30]. Iontophoresis of DA or its antagonists have been found to change the spontaneous activity of both on-center and off-center ganglion cells or only off-center cells. DA antagonists potent at D_1 receptors can reduce or abolish the response of antagonist surround in rabbit retinal ganglion cells while more specific D_2 antagonists had little effect[11]. A rapid desensitization in DA-sensitive adenylate cyclase activity, in response to light, may be one way in which dopaminergic neurons modulate the sensitivity of ganglion cells to light. This method would provide a quick and effective modulation of ganglion cell input that would not necessitate a change in receptor number. Direct synapses from dopaminergic amacrine cells onto ganglion cells have not been identified. In cat retina, however, dopaminergic amacrine cells synapse on AII amacrine cells[23] which output directly on off-center ganglion cell dendrites[13,14]. Therefore dopaminergic neurons may exert their influence on ganglion cells indirectly through the AII amacrine cells by a D_1 receptor-mediated response that is under tight dopaminergic regulation.

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