The inflammatory response to trauma in the eye may be mediated by neurogenic factors, conceivably through axon reflexes (*2, 10*). Cholinergic mechanisms do not seem to be involved (*2*). Prostaglandins have been proposed as mediators of inflammatory responses to certain ocular trauma, such as anterior chamber paracentesis and laser irradiation of the iris (*20*). Conceivably also prostaglandins act via neurogenic mechanisms; an intact sensory innervation of the eye is a prerequisite for prostaglandin-evoked responses in the rabbit eye (*21*). Intracameral injection of SP evokes effects similar to those seen after acute trauma to the eye (*5*). Therefore, since SP exists in nerve fibers of the uvea (*12, 13*), SP may be one of the anticipated neurogenic mediators of the vasodilatation, disruption of the blood-aqueous barrier, and miosis associated with inflammation in the eye. Intravitreal application of [D-Pro^2, D-Trp^{7,9}]SP greatly reduced not only the effects of exogenous SP but also the inflammatory response to trauma to the eye. Since [D-Pro^2, D-Trp^{7,9}]SP is a specific SP antagonist (*16*), these observations support the view that SP is one of the neurogenic mediators of the inflammatory response [see (*22*)], and moreover suggest a possible clinical use of SP antagonists in alleviating inflammatory symptoms in the eye, particularly since topical application was sufficient to reduce inflammation. The corneal sensitivity seemed unaffected by treatment with the SP antagonist, suggesting that the sensory afferents in the cornea do not depend upon SP for nociception (*13, 23*).

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Dopamine Receptor Binding Is Increased in Diabetic Rats

Abstract. *The binding of [^{3}H]spiperone, a dopamine receptor ligand, to striatal membranes was increased 30 to 35 percent in rats made diabetic with alloxan or streptozotocin. Binding of [^{3}H]spiperone was normal in rats made diabetic with alloxan but treated with insulin. Thus the number of dopamine receptors and central dopaminergic transmission may be altered in diabetes.*

We previously found that glucose administration rapidly and completely suppresses the firing of dopamine (DA)-containing neurons innervating the rat striatum (*1*). Since DA receptor sensitivity can be increased by treatments that cause sustained reductions in the concentration of intrasynaptic DA (*2*), it is of interest to examine the effects of chronic hyperglycemia on DA receptor sensitivity. We now report the effect of diabetes induced by alloxan or streptozotocin on the binding of [^{3}H]spiperone to membranes prepared from striatal tissues. The in vitro binding of [^{3}H]spiperone appears to provide a reliable index of DA receptor sensitivity (*3*). An examination of DA receptor binding in diabetic animals is also desirable in view of the evidence implicating changes in the function of dopaminergic neurons in the etiology of behavioral and mood disorders (*4*) and emotional disturbances sometimes associated with diabetes (*5*).

Male Sprague-Dawley rats (Zivic-Miller) weighing 200 to 275 g were housed in groups of five to six per cage. They were provided with unrestricted quantities of Purina Rat Chow and tap water and kept in a room with 12-hour cycles of light (600 to 1800 hours) and darkness.

In an initial experiment, the rats were injected subcutaneously with alloxan monohydrate dissolved in 0.9 percent saline (200 mg/kg; $N = 15$) or with 0.9 percent saline (2 ml/kg; $N = 16$). Both groups were decapitated at 1000 to 1400 hours 6 weeks later. Striata were dissected on ice, immediately frozen on dry ice, and stored at −80°C until being analyzed for [^{3}H]spiperone binding. Five pools of striatal tissues, each from two or three diabetic rats, and six pools of tissues, each from two or three controls, were assayed in triplicate with five concentrations of spiperone (0.1 to 2.4 n*M*) (*6*). Nonspecific binding was assessed by assaying a second set of samples in the presence of a saturating concentration (10 μ*M*) of the DA receptor antagonist (+)-butaclamol. Specific binding was defined as the difference in [^{3}H]spiperone binding in the presence and absence of (+)-butaclamol. The maximum specific binding and dissociation constants were calculated from least-squares fits of Scatchard plots of the binding data (*7*). Blood glucose concentrations were also determined for each animal (*8*).

Blood glucose was greatly elevated in all of the alloxan-treated rats (Table 1), indicating the effectiveness of the alloxan treatment (*9*). Maximal spiperone binding was 30 percent greater in the alloxan-treated rats ($P < .01$, Student's *t*-test), but the dissociation constant for spiperone was not altered (Table 1). Thus the number of striatal DA receptors appears to increase in alloxan-treated rats (*10*).

In a second experiment, diabetes was produced by administering streptozotocin (*11*). Six rats received intraperitoneal injections of streptozotocin (75 mg/kg) and six received saline (2 ml/kg). All the

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Table 1. Binding of [^{3}H]spiperone to striatal membranes from diabetic and control rats. Body weight changes in these experiments were similar to those reported in Table 2. Values are means ± standard errors.

Treatment	*N**	[^{3}H]Spiperone binding (fmole/mg-protein)	Dissociation constant (n*M*)	Blood glucose (mmole/liter)
		Experiment 1 (pooled striata)		
Saline	6	223.0 ± 13.4	0.20 ± 0.06	5.82 ± 0.16
Alloxan	5	289.0 ± 9.4†	0.24 ± 0.04	19.78 ± 0.85‡
		Experiment 2 (individual striata)		
Saline	6	154.5 ± 20.0		7.19 ± 0.05
Streptozotocin	6	209.2 ± 11.9§		22.40 ± 4.94‡

*Number of determinations. †Significantly different from associated control value at $P < .010$ (Student's *t*-test). ‡$P < .001$. §$P < .025$.

Table 2. Effects of insulin therapy on [^{3}H]spiperone binding, weight gain, and blood glucose concentrations in diabetic and control rats. Values are means ± standard errors.

Treatment	*N**	[^{3}H]Spiperone binding (fmole/mg-protein)	Weight gain (g)	Blood glucose (mmole/liter)
Saline + saline	7	113.7 ± 10.7	215 ± 35	6.14 ± 0.30
Alloxan + saline	7	157.4 ± 11.3†	117 ± 18†	22.92 ± 1.67‡
Saline + insulin	6	117.2 ± 8.4	283 ± 13	7.88 ± 1.29
Alloxan + insulin	7	121.0 ± 12.8	237 ± 19	4.71 ± 0.70

*Number of rats. †Significantly different from the other three values at $P < .05$ (Newman-Keuls test). ‡$P < .01$.

animals were decapitated 6 weeks after treatment. The number of DA receptors in each rat was estimated by measuring the binding of a saturating concentration (1.2 n*M*) of spiperone to striatal membranes prepared from individual animals. The concentration of glucose was measured in duplicate portions of trunk blood. The streptozotocin-treated rats exhibited moderate to severe hyperglycemia and appeared to have more DA receptors in the striatum than the controls, as evidenced by the greater binding of [^{3}H]spiperone to striatal membranes (Table 1).

Next, we examined the effects of insulin administration on the binding of [^{3}H]spiperone to striatal membranes. Rats were injected subcutaneously with alloxan monohydrate (185 mg/kg; $N = 14$) or saline (2 ml/kg; $N = 13$). Beginning 30 days later, seven alloxan-treated rats and six saline-treated rats were given two subcutaneous injections of protamine zinc insulin (Lilly) daily for 12 days. The first injection was given at the beginning of the light period and the second near the end of the light period (2 U/kg per injection on days 1 and 2 and 8 U/kg per injection thereafter). All the other animals received two saline injections daily. On the day after the last insulin or saline injection, all animals were decapitated within 2 hours of the midpoint of the light period. Striata were removed and the binding of 1.2 n*M* [^{3}H]spiperone to striatal membranes from individual rats was measured. The concentration of glucose was determined in trunk blood collected into tubes containing EDTA. The body weight of each animal was also monitored to assess the effectiveness of the insulin treatment.

As before, alloxan treatment increased blood glucose concentrations and [^{3}H]spiperone binding in the striatum (Table 2). Insulin treatment normalized blood glucose. It also promoted weight gain in the diabetic rats (Table 2), indicating the adequacy of the treatment in suppressing alloxan-induced hyperglycemia throughout the treatment period (*9, 12*). Most important, in the alloxan-treated rats given insulin (*13*), [^{3}H]spiperone binding was similar to that in controls (Table 2). Insulin given to normal rats did not alter [^{3}H]spiperone binding (*14, 15*). Regression analysis of the data obtained from all the animals in this experiment revealed that [^{3}H]spiperone binding was positively correlated with blood glucose concentrations ($r = .61$, $P < .01$) and negatively correlated with the amount of weight gained during the experiment ($r = .63$, $P < .01$). Thus, under these conditions, the number of DA receptors in diabetic animals may be related to the severity of the diabetes.

In summary, [^{3}H]spiperone binding is increased in both alloxan- and streptozotocin-induced diabetes in rats. These increases are comparable to those observed after long-term blockade of DA receptors or after lesions of striatal DA-containing neurons (*3*). Since [^{3}H]spiperone binding appears to reflect DA receptor sensitivity (*3*), these findings strongly suggest that the sensitivity of these receptors is substantially increased in diabetic rats. Moreover, [^{3}H]spiperone binding was normal in insulin-treated diabetic rats, suggesting that insulin normalizes DA receptor sensitivity in diabetic rats. Increases in DA receptor sensitivity and dopaminergic transmission have been implicated in the pathogenesis of schizophrenia (*4*), and repeated insulin-induced coma was used with some success in treating schizophrenia (*16*) before the introduction of neuroleptics. The present observations are consistent with the hypothesis that the behavioral effects of hypoglycemia are mediated by changes in DA receptors.

It is well known that decreases in DA release can lead to increases in DA receptor sensitivity (*2*). We have reported that the firing of DA-containing neurons that project from the substantia nigra to the striatum is rapidly suppressed when blood glucose is elevated (*1*). Thus, long-term hyperglycemia in diabetic animals may lead to chronic hypofunction of central DA-containing neurons. Consistent with this hypothesis, amphetamine-induced anorexia, locomotion, and stereotypy—behaviors dependent on the functional capacity of central dopaminergic neurons—are diminished in rats with alloxan diabetes (*17*). Moreover, insulin treatment restores the behavioral responsiveness to amphetamine in diabetic rats (*17*). Increased DA receptor sensitivity may be a compensatory adjustment to a reduction in central dopaminergic neuronal activity. Investigations of DA turnover and release in diabetic animals may clarify this issue (*18*).

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13. Preliminary studies indicate that daily administration of protamine zinc insulin (4 U per rat, subcutaneously), initiated 5 days after alloxan treatment, also prevents the increase in [^{3}H]spiperone binding observed 6 weeks after alloxan injection.
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Correcting the Phenotype of the Epidermis from Chick Embryos Homozygous for the Gene Scaleless (*sc/sc*)

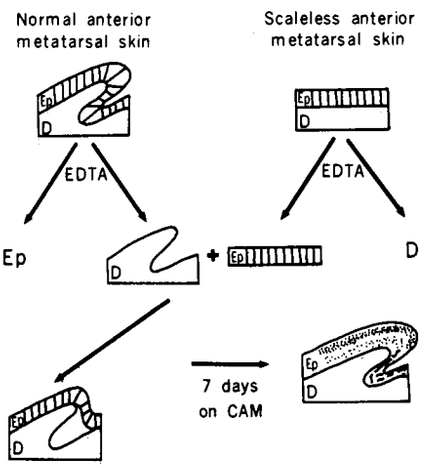
Abstract. *Scutate scales are completely missing in the scaleless* (sc/sc) *mutant chicken. Organ cultures consisting of epidermis from* sc/sc *embryos combined with normal (+/+) scale dermis of the same developmental age produce the scaleless phenotype, but the same scaleless epidermis in combination with normal dermis from more differentiated embryonic scales forms perfectly normal scales.*

The epidermal and dermal components of the avian skin undergo inductive tissue interactions that result in the morphogenesis of feathers and scales (*1, 2*). The scaleless mutant chicken (*3*) carries a recessive, autosomal mutation which, in the homozygous state (*sc/sc*), results in the lack of most of the feathers on the body and all of the scutate scales that are located along the anterior metatarsi. Biochemical, x-ray diffraction, and fine structural studies comparing normal scutate scale epidermis and the scaleless epidermis show that the beta stratum (made up of beta-type keratins), which characterizes the hard, platelike surface of the normal scutate scale, is totally missing from the scaleless epidermis (*4–8*).

Scutate scales first appear as discrete epidermal thickenings (scale placodes) along the anterior metatarsal surface of the legs and feet at 10 days of incubation (stage 36). Reciprocal epidermal-dermal tissue recombinations between normal and mutant anterior metatarsal skin at stages 36 and 37 have demonstrated that the scaleless defect is expressed initially by the embryonic epidermis (*9, 10*). Tissue recombination studies further show that the scaleless dermis itself becomes defective as development progresses (*11, 12*). It is not until stage 38, 2 days after initial scale placode formation, that the

Table 1. Development of overlapping scutate scales in recombinant grafts between scaleless anterior metatarsal epidermis (stage 36 to 42) and stage 40, 41, or 42 normal anterior metatarsal dermis cultured for 7 days on chick chorioallantoic membrane.

Stage of scaleless epidermis	Recombinant grafts		Macroscopic appearance of recovered recombinant grafts	
	Number done	Number recovered	With scales	Without scales
36	5	2	2	0
37	2	1	1	0
38	4	4	4	0
39	11	6	6	0
40	25	17	17	0
41	20	14	14	0
42	12	10	9	1



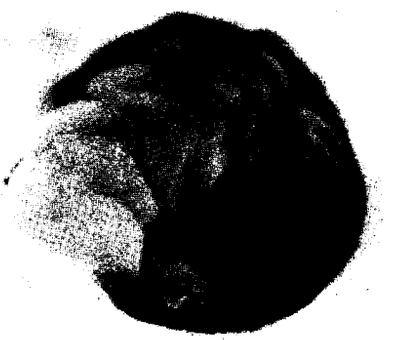


Fig. 1 (left). Diagram of the tissue recombination experiments between scaleless anterior metatarsal epidermis and normal scutate scale dermis. After 7 days of growth on chick chorioallantoic membrane these grafts developed typical scales with an outer epidermal surface (dots) and an inner epidermal surface (wavy lines). Abbreviations: *Ep*, epidermis; *D*, dermis; *CAM*, chorioallantoic membrane. Fig. 2 (right). Photograph showing the scales formed from scaleless anterior metatarsal epidermis recombined with normal scutate scale dermis and grown for 7 days on chick chorioallantoic membrane (×35); *SS*, scutate scales.

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