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Norepinephrine and epinephrine levels in the brain of alloxan diabetic rats

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Abstract

Thirty days after induction of experimental diabetes the brain catecholamines namely, norepinephrine (NE) and epinephrine (E) were studied in discrete brain regions (striatum, hippocampus, hypothalamus, midbrain, pons and medulla, cerebellum and cerebral cortex) in control, alloxan-diabetic untreated and insulin-treated diabetic rats. E showed significant increase in striatum, hippocampus and hypothalamus, whereas NE was increased in hypothalamus, and decreased in pons and medulla significantly in untreated diabetic rats. These effects were not seen in the insulin-treated diabetic rats.

Keywords: Diabetes; Alloxan; Insulin; Norepinephrine; Epinephrine; Rat brain

Uncontrolled diabetes is associated with a significant disturbance of brain monoamine metabolism [1] and even with a short period of uncontrolled diabetes central neurochemical alterations occur [18]. These alterations were reversed when diabetic rats received insulin replacement therapy [3,11]. Experimental diabetes in rats was accompanied by an increase in the levels of norepinephrine (NE) and dopamine [10] in many brain regions. The turnover rate of brain NE decreases in diabetic animals [17]. NE concentration was considerably reduced in the cardiovascular system of diabetic patients [14]. Mouse brain NE showed increased levels after 3, 50 and 100 days (diabetic) in some specific regions [2]. In another study [16], the NE concentration was elevated in cerebral cortex, cerebellum and medulla oblongata in diabetic rats. The epinephrine (E) level showed significant increase in striatum and decrease in hypothalamus after 2 weeks in diabetic animals [3].

However, a perusal of the literature has shown that only certain regions of the rodent brain have been studied in these previous works and information on the alteration of monoamines in all the discrete areas of rat brain is not available. Hence, in the present study, we examined the level of NE and E in discrete areas of brain in alloxan-induced untreated diabetes, as well as in insulin-treated diabetic animals along with controls.

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Wistar strain male albino rats (150–200 g) were group-housed and maintained under standard laboratory conditions. Overnight starved rats were made diabetic by a single injection of alloxan (45 mg/kg i.v.) dissolved in 0.9% saline as described by Korec [15] and left untreated for 30 days. In another group, rats injected with alloxan (45 mg/kg i.v.) were treated with zinc insulin (5 IU/kg) subcutaneously daily for 30 days to maintain blood sugar within the normal range. Control animals received saline only. All the experimental animals were fed with food and water ad libitum. Diabetes was verified by hyperglycemia and glucosuria. In both the control and test group animals, blood sugar was monitored at weekly intervals by the *o*-toluidine method [4]. Urine sugar was monitored daily using Benedict's reagent.

Thirty days after the induction of alloxan diabetes, control, diabetic untreated rats and diabetic rats treated with insulin were decapitated between 0900 and 1000 h to avoid circadian influences on brain catecholamines. Brains of these animals were rapidly removed and dissected into seven regions, weighed and homogenized [7]. After butanol extraction, NE and E were determined spectrofluorimetrically (Hitachi model 650-10 M, fluorescence spectrophotometer) by the method described by Kari et al. [9].

All the results obtained were analyzed by one-way analysis of variance (ANOVA) and if there was a significant F ratio, the results were further analyzed by Tukey's multiple comparison test.

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The alloxan-diabetic untreated rats showed a higher glucose level, ranging from 240 to 300 mg% with ++++ sugar in the urine. Insulin-treated rats maintained normal blood glucose levels (70–100 mg%) and no sugar was detected in the urine. The concentration of NE and E in discrete areas of brain in different groups after 30 days treatment are presented in Table 1.

The E level increased significantly in striatum and hypothalamus ($P < 0.001$) and in hippocampus ($P < 0.05$) in alloxan diabetic rats. Whereas NE showed a significant increase in hypothalamus ($P < 0.01$) and decrease in pons and medulla ($P < 0.01$) compared to control animals. Insulin-treated diabetic rats showed no significant change in any of the areas studied compared to controls.

The rationale of using alloxan in the present study is that it selectively destroys the beta cells and so produces a diabetic state very similar to spontaneous diabetes in man. Further, alloxan is capable of producing diabetes in every species when appropriate doses and procedures are used and the technique of producing diabetes in the rat which has a diffuse pancreas is relatively easy with this agent compared to pancreatectomy. The severity of diabetes can also be graded by administration of different doses of alloxan. Based on our preliminary work, animals with 30 days of diabetes were used in this study as this duration is taken as the subacute stage in the course of alloxan diabetes. A longer duration of diabetes may induce severe metabolic alterations and a shorter duration may not have produced all the metabolic readjustments in established experimental diabetes (unpublished observations).

The observations in the present study demonstrate that alloxan-induced diabetes after 30 days, results in a significant increase in the content of E in striatum, hippocampus and hypothalamus. The NE level showed a significant increase in hypothalamus and a decrease in pons and medulla. These catecholamine changes were not observed in other areas or in rats receiving insulin replacement therapy.

The increased NE content in the hypothalamus in our study in rats was similar to other reports in spontaneously diabetic mice, rats and Chinese hamsters [2,6,10,12]. However, Chu et al. [3] found a decreased hypothalamic NE level in diabetic rats after 2 weeks. The mechanism responsible for the elevation of hypothalamic NE levels is not very clear. Several possibilities can be considered [1,11,17]: inhibition of presynaptic release of NE and/or an increased re-uptake of released NE could lead to accumulation of NE. Alternatively, a decrease in the metabolic degradation of NE could also be involved. The NE level in pons and medulla showed a significant decrease after 30 days in this study, which is not in agreement with other reports. There was an increased level of NE in pons and medulla in diabetic animals [2,5,8,16], whereas other reports [1,18] showed no significant change in pons and medulla.

A decrease in the steady state level of NE in pons and medulla could be due to either decreased synthesis or an increased rate of degradation. Further studies are needed to clarify whether the observed changes in NE level were due to altered turnover rate.

On the other hand, the present results show that the untreated diabetic animals had a higher level of E in striatum, hippocampus and hypothalamus, while other areas (mid brain, pons and medulla, cerebellum and cerebral cortex) showed no change. These findings differ from the previous work of Chu et al. [3], who reported the striatal E level was significantly elevated and hypothalamic E was significantly decreased after 2 weeks in diabetic animals. Tasaka et al. [16] reported no change in E level in hypothalamus of diabetic animals. The cause of

Table 1

Norepinephrine and epinephrine levels (ng/g of wet tissue) in discrete regions of the brain in control, alloxan diabetics and insulin-treated diabetic rats

	Striatum		Hippocampus		Hypothalamus		Midbrain		Pons and medulla		Cerebellum		Cerebral cortex	
	NE	E	NE	E	NE	E	NE	E	NE	E	NE	E	NE	E
Control rats ($n = 15$)	335 ±17	145 ±12	222 ±13	125 ±6	1367 ±35	206 ±17	527 ±15	297 ±24	610 ±30	210 ±10	161 ±10	103 ±9	226 ±14	99 ±8
Alloxan diabetic rats ($n = 6$)	314 ±19	245 ±14	285 ±25	183 ±25	1724 ±113	501 ±26	560 ±33	302 ±46	370 ±40	265 ±40	172 ±39	100 ±14	285 ±60	112 ±20
Insulin-treated diabetic rats ($n = 6$)	340 ±17	110 ±3	210 ±19	104 ±14	1394 ±51	244 ±37	551 ±19	264 ±33	612 ±47	222 ±16	151 ±10	104 ±17	213 ±24	97 ±11
Significance														
1	NS	<0.001	NS	<0.05	<0.01	<0.01	NS	NS	>0.01	NS	NS	NS	NS	NS
2	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
3	NS	<0.001	NS	0.01	<0.01	<0.001	NS	NS	>0.01	NS	NS	NS	NS	NS

Values are given as mean ± SEM. 1, control versus alloxan diabetic untreated; 2, control versus insulin-treated diabetic; 3, alloxan diabetic untreated versus insulin-treated diabetic; NS, not significant. n, the number of animals used.

the discrepancies in the results of various workers is difficult to explain. One important fact to be considered in this connection is the sensitivity of the central nervous system (CNS) to various diabetogenic agents. It has been suggested that brain catecholamines are regulated by factors within the CNS itself and independent of peripheral glucose level. It is quite possible that a specific region or center in the brain is vulnerable to the diabetogenic effects of alloxan and streptozotocin and is a central point controlling brain catecholamines [10]. Based on this obervation, it could be possible that the discrepancies in the levels of various amines in the brain of diabetic animals reported by various authors could be due to the different diabetogenic agents used. In addition, the physiological state of the region concerned at the time of drug administration/sacrifice could also influence the outcome of the results.

Although the exact physiological significance of the alterations in E and NE in hypothalamus and other areas is not known, it may be suggested that as these amines are related to food intake and satiety, such a change may be directed towards establishing appropriate energy intake by the diabetic animals [13].

From scrutiny of the results, it is evident that different regions of the brain have differential responses to experimental diabetes, which is also amine specific. In this connection, it is pertinent to point out that alteration of a particular amine in a particular region could be due to the diabetic state (lack of insulin) per se or it could also be due to other metabolic consequences of diabetes mellitus contributing to the genesis of these changes. Hence, the differential sensitivity of different regions of the brain could be responsible for the variable results obtained for a particular amine.

Thus, the present study has yielded further support for the previous reports on monoamine alteration in the diabetic state. The elevated E and NE concentrations in the hypothalamus may be responsible for altered food intake seen in diabetic individuals. The depressed NE levels in pons and medullary regions may contribute to autonomic impairment. Further studies are necessary to elucidate the physiological significance of these findings and speculations. Further, the fact that all these changes in brain biogenic amines did not manifest themselves in insulin-treated diabetic rats, indicate that the effects observed are due to the diabetic state.

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