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ALPHA₂ ADRENERGIC AND HIGH AFFINITY SEROTONERGIC RECEPTOR CHANGES IN THE BRAIN STEM OF STREPTOZOTOCIN-INDUCED DIABETIC RATS

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Summary

The brain stems (BS) of streptozotocin (STZ)-diabetic rats were studied to see the changes in neurotransmitter content and their receptor regulation. The norepinephrine (NE) content determined in the diabetic brain stems did not show an increase, while epinephrine (EPI) content increased significantly compared with control. The NE to EPI turnover showed a significant increase. The alpha₂ adrenergic receptor kinetics revealed that the receptor affinity was significantly reduced during diabetes. In insulin treated rats the NE content decreased while EPI content remained increased as in the diabetic state. Insulin treatment increased the B_{max} for alpha₂ adrenergic receptors significantly while the increase in K_d reversed to normal. Unlabelled clonidine inhibited [³H]NE binding in BS of control, diabetic and insulin treated diabetic rats showed that alpha₂ adrenergic receptors consisted of two populations of binding sites with Hill slopes significantly away from unity. In diabetic animals the ligand bound weaker to the low affinity site than in controls. Insulin treatment reversed this alteration to control levels. The displacement analysis using (-)-epinephrine against [³H]yohimbine in control and diabetic animals revealed two populations of receptor affinity states. In control animals, when GTP analogue added with epinephrine, the curve fitted for a single affinity model; but in the diabetic BS this effect was not observed. In both the diabetic and control BS the effects of monovalent cations on affinity alterations were intact. Our data thus show that alpha₂ adrenergic receptors have a reduced affinity due to an altered post receptor affinity regulation. The serotonin (5-HT) content in the brain stem increased. Its precursor (5-hydroxy) tryptophan (5-HTP) showed an increase and its breakdown metabolite (5-hydroxy) indoleacetic acid (5-HIAA) showed a significant decrease. This showed that in serotonergic nerves there is a disturbance in both synthetic and breakdown pathways which lead to an increased 5-HT. The high affinity serotonin receptor numbers remained unaltered with a decrease in the receptor affinity. The insulin treatment reversed these altered serotonergic receptor kinetic parameters to control level. Thus our study shows a decreased serotonergic receptor function. These changes in adrenergic and serotonergic receptor function were suggested to be important in insulin function during STZ diabetes.

Key Words: brain stem, streptozotocin-diabetes, adrenergic receptors, serotonergic receptors

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The pancreatic secretion of insulin is under the direct control of central nervous system (CNS) inputs from the lower brain regions. The adrenergic and serotonergic nervous system input from the brain stem (BS) was shown to innervate the vagal motor nerve (1). The streptozotocin (STZ) diabetes was shown to be associated with large alterations in brain monoamine contents (2,3). The central serotonergic_{1A} (5-HT_{1A}) receptor agonist 8-hydroxy-2-di-n-propylamino tetralin (8-OH-DPAT), was shown to induce hyperglycemia in normal rats (4). The effect of this drug was shown to be blocked by alpha₂ adrenergic receptor antagonist (5). This showed that serotonin (5-HT) receptors bring about this effect together with alpha₂ adrenergic receptors(6).

In STZ-diabetic BS the 5-HT_{1A} and 5-HT₂ receptor numbers were shown to be altered (7). The studies so far done to show the roles of serotonergic receptor changes in hyperglycemic conditions have put less emphasis on an adrenergic component. It has been shown that diabetes causes changes in the adrenergic nerves in brain regions (8-11). A role for serotonergic and adrenergic components in the regulation of glucose homeostasis prompted us to look into the alpha₂ adrenergic and serotonergic nerve activity in brain stem of diabetic rats. In several earlier studies adrenergic activity assessed by NE and its metabolite EPI have been used to demonstrate an involvement of alpha₂ adrenergic receptors (12,13). In the present study we analyzed changes in the NE, EPI and kinetics of alpha₂ adrenergic receptors in STZ-diabetic rats. Alpha₂ adrenergic receptors were further analyzed for their second messenger regulated affinity changes during diabetic state. We report here a shift in the affinity status of alpha₂ - adrenergic receptors and this change in affinity was shown to be due to a changed nucleotide mediated affinity regulations. We also analyzed serotonergic activity by measuring its content and receptor kinetics. An increased serotonergic content was found to be associated with a reduced affinity for high affinity serotonergic receptors.

Methods

Drugs

1-[7,8-³H]noradrenaline, 39.0 Ci/mmol, [O-methyl-³H]yohimbine, 88 Ci/mmol and (5-hydroxy) [G-³H] tryptamine creatinine sulfate, 18.4 Ci/mmol were bought from Amersham, UK. The streptozotocin neurotransmitter standards, other receptor agonists and 5'-guanylimido-diphosphate-trisodium salt (Gpp[NH]p) were purchased from Sigma chemical company, St. Louis, MO, USA. Clonidine was a gift from Boehringer, Ingelheim, Germany.

Animal experiments

Adult male Sprague-Dawley rats of 6-8 months of age and ~200g body weight was used for all experiments. The streptozotocin was given as i v injections (45-50 mg/ kg body weight). The rats were kept under constant lighting conditions (12-hr light/12 hr dark cycle) throughout the experiments. The food and water were given *ad libitum*. The control animals were given the citrate buffer and kept under the same conditions. The diabetic animals were randomly divided into two groups and one group was given insulin injections (2-6 units) during the last two weeks. The last injections were given 24 hr. prior to the killing. At the end of a period of 28 days the animals were killed by decapitation. The brain was dissected into different region (14) and were immediately immersed in liquid nitrogen and stored at -70°C till used for further experiments.

Monoamine analysis

The monoamines were estimated using high performance liquid chromatography (HPLC, Shimadzu, Japan) fitted with a C18-ODS reverse phase column (15). The

diamine tetra acetic acid and 8% acetonitrile, pH 3.2. The catecholamines were identified by amperometric detection using an electrochemical detector (ECD) at a reduction potential of 0.8V. Twenty μ L of 0.4N perchloric acid extract of brain stem tissues was injected into the system. The peaks were identified by relative retention times compared using an integrator interfaced with the ECD.

Receptor assays using [3 H] radioligands

Alpha₂ adrenergic receptor assay using [3 H] yohimbine binding studies were done according to Repaske *et al.*, (16). The content of protein per assay mixture used was within range of 0.2 to 0.4 mg. The incubation was done for 90 minutes at 15°C and stopped by filtration through GF/C filters followed by three washes of 5 ml each of ice cold buffer containing 25 mM glycylglycine pH 7.6. The binding extents maximal binding (B_{max}) and dissociation constant (K_d) were calculated from Scatchard plots(17). The serotonin receptor analysis was done as previously reported (18). The experiments to determine receptor affinities for alpha₂ receptors were performed as reported earlier (16,19,20). In experiments to analyze the alpha₂ adrenergic affinity the sodium ion concentrations were kept constant by including 100 mM NaCl in the buffer.

Receptor data analysis

The receptor data was analyzed by iterative nonlinear regression using a computer program Prism, Graph Pad Software, Inc. USA. The concentration of competitor that competes for half the specific binding was defined as EC_{50} . It is same as the IC_{50} (21). The affinity of the receptor for the competing drug is designated as the K_i and is defined as the concentration of the competing ligand that will bind to half the binding sites at equilibrium in the absence of radioligand or other competitors (22). The data were analyzed using Student's t-test and ANOVA (23).

Blood glucose and protein estimations

The blood glucose was assayed using a glucose oxidase enzyme kit (Merck, India). The protein was estimated by the method of Lowry *et al.*, (24), using bovine serum albumin as standard.

Results

Induction of diabetes with streptozotocin elevated the blood glucose level significantly (550 ± 18 mg /dl in diabetic Vs 87 ± 10 mg / dl in control). The body weights in diabetic rats compared to controls were significantly reduced (control final body weight 182 ± 20 gms; diabetic final body weight 140 ± 5 gms). The insulin treatment reversed the body weight near to normal (170 ± 8 gms). The water uptake in both diabetic and insulin treated diabetic rats (D+I rats) was significantly elevated compared to the controls (190 ± 22 ml /day in diabetic and 100 ± 8 ml /day in D+I rats Vs 30 ± 8 ml/ day in control rats).

The neurotransmitter contents estimated in the brain stem of control, diabetic and D+I rats were given in Table I. The NE content in diabetic brain stem showed no change while insulin treated rats showed significantly reduced NE content ($p < 0.05$). The EPI content in both diabetic and D+I rats was significantly elevated ($p < 0.01$). The serotonin content showed a significant increase in diabetic rats ($p < 0.05$). The insulin treatment was able to reverse this altered parameter. The precursor of serotonin, 5-HTP showed a significant increase ($p < 0.05$)

while its metabolite 5-HIAA decreased significantly ($p < 0.05$) in diabetic rats. The effect of insulin on 5-HTP and 5-HIAA was that 5-HTP decreased ($p < 0.05$) while 5-HIAA remained the same as in the diabetic rats ($p < 0.05$).

The turnover ratio calculated for the neurotransmitters to their respective metabolites in BS showed that NE to EPI turnover increased in diabetes significantly ($p < 0.01$; Table I). In insulin treated rats also the ratio of EPI to NE remained significantly elevated ($p < 0.01$). The 5-HTP to 5-HT turnover rate showed a significant increase ($p < 0.05$) while 5-HT to 5-HIAA turnover showed a significant decrease ($p < 0.01$). With insulin treatment the turnover ratio of 5-HTP to 5-HT was decreased ($p < 0.05$) compared to both control and diabetic rats. In D+I rats the ratio of 5-HT to 5-HIAA significantly decreased compared to control ($p < 0.01$).

[³H]yohimbine receptor Scatchard analysis in brain stem of diabetic rats showed no change in the B_{max} but a significant increase in the K_d ($p < 0.05$; Table II). This showed that α_2 adrenergic receptors of diabetic brain stem have a significantly reduced affinity, but no change in the total number of receptors. Diabetic rats treated with insulin showed a significantly elevated B_{max} and no change in K_d . The insulin reversed the changed receptor affinity but receptor numbers were elevated significantly ($p < 0.05$).

The competition curve for unlabelled clonidine inhibited specific [³H]NE binding fitted for a two site binding model in control, diabetic and D+I rats. In diabetic rats the low affinity state value increased when compared to the control values. This indicates that the affinity decreased for the low affinity state (Table III). Insulin treatment reversed these altered parameters to control level.

The effect of guanine nucleotides and sodium ions was assessed in control and diabetic brain stem to see whether the observed reduced affinity of α_2 adrenergic receptors was a result of changed nucleotide or ion mediated affinity regulations. The data were analyzed using a two-site Vs one-site binding model. Our data in control BS with epinephrine competed for [³H]yohimbine binding fitted for a model for two sites binding. The Hill slope for this curve was significantly far from unity (0.3026; Table IV). When GTP analogue was added, the curve shifted to a lower affinity and the curve fitted for a one-site binding model. The Hill slope was closer to unity with a value 0.7006. When sodium ions added, the curve also fitted for a single-site binding model with slope factor closer to unity (0.8704). The curve for the combined effect of both GTP analogue and sodium ions added with epinephrine fitted for a single-site binding model with a Hill-slope factor of 0.8533. In epinephrine competed curve, the curve descended from 90 to 10 percent specific binding over a hundred fold change in concentration of competing ligand. When GTP analogue added, the same change was observed within a much smaller concentration range of the competing ligand (less than 50 fold). These results suggest that in control BS G protein regulated and sodium ions regulated receptor affinity changes are intact.

In diabetic BS the epinephrine inhibited binding of [³H]yohimbine fitted for a two-site model with more affinity for the low affinity site. The Hill slope for this curve is closer to unity (0.7001). When GTP analogue was added the curve fitted for a two-site model with a decrease in affinity of ligand for the high affinity sites. (Table V). The Hill slope for this curve was significantly away from unity (0.6236). In the experiments to see the effect of sodium ions on receptor affinity the Hill slope was unity with a single site binding curve. The curve for both compounds added together best fitted for one site model.

In both control and diabetic animals the value for K_i-1 (affinity for high affinity site) was lower when compared to K_i-2 (affinity for the high affinity site) indicating a stronger

TABLE I
Neurotransmitters and their metabolites and turnover rates in the brain stem of control, diabetic and diabetic with insulin treated rats
(nanomoles /gram wet weight)

	NE	EPI	EPI/NE ^a	5-HT	5-HTP	5-HTP/5-HT ^a	5-HIAA	5-HIAA/5-HT ^a
Control	2.84±0.26	0.08±0.01	0.04±0.01	0.67±0.13	0.37±0.04	0.54±0.09	3.64±0.53	4.25±0.75
Diabetic	3.45±0.30	0.26±0.07 **	0.10±0.04 **	1.71±0.11 *	1.28±0.15 *	0.74±0.07 *	1.49±0.45 *	1.18±0.37 **
Diabetic with insulin	1.85±0.15 *	0.23±0.07 **	0.12±0.02 **	0.78±0.15	0.19±0.04 *	0.24±0.01 *	1.44±0.40 *	1.89±0.33 **

*0.05 compared to control
 **0.01 compared to control
 Values are from diabetic rats (artificially induced over a 28-day period)
 value represents averages from 4-6 separate animal experiments
^aNE, 5-HTP/5-HT and 5-HIAA /5-HT are ratios
 of norepinephrine, EPI- Epinephrine, 5-HT- (5-hydroxy)tryptamine
 P-(5-hydroxy)tryptophan,
 AA-(5-hydroxy)indoleacetic acid

binding to Ki-1. In control BS when GTP analogue added the affinity shifted to a single affinity with a significant increase in the affinity for Ki-2 site in control. In diabetic BS there is no shift to a single affinity observed when GTP analogue added. The value for Ki-2 showed an increase compared to control BS and Ki-2 of epinephrine alone added conditions in diabetic BS itself. In control BS the effect of NaCl and NaCl + Gpp[NH]p on the value of Ki-2 was that it decreased when compared to Ki-2 value for Epi alone and Epi + Gpp[NH]p cases, which shows an increase in the affinity (Table IV). In the case of diabetic animals value of Ki-2 in the NaCl and NaCl + Gpp[NH]p groups increased compared to control groups, yet the affinity shifted to a single low affinity (Table V).

Epinephrine when competed for [³H]yohimbine binding the Ki-2 was higher than Ki-2 from control brains (Table IV and V). This result is not the same as the one observed with clonidine competed for [³H]NE binding, where the Ki-2 in diabetic animals is lower than Ki-2 in control (Table III).

[³H]5-HT Scatchard in BS showed that B_{max} remained same and K_d increased significantly in diabetes (P < 0.05; Table II). Insulin treatment reversed these altered parameters to control levels.

Table II
Binding parameters of Alpha₂ Adrenergic and Serotonergic Receptors in Brain stem

Animal Status	[³ H] yohimbine		[³ H]serotonin	
	B _{max} (fmoles/mg of protein)	K _d (nM)	B _{max} (fmoles/mg of protein)	K _d (nM)
Control	228 ± 15	5.56 ± 0.02	197 ± 2	1.24 ± 0.02
Diabetic	269 ± 28	8.85 ± 0.01*	222 ± 12	4.62 ± 0.12*
Diabetic + Insulin	327 ± 30*	6.55 ± 0.01	185 ± 3	1.31 ± 0.40

*p < 0.05 compared to control

B_{max} - Maximal binding, K_d - Dissociation constant

Discussion

The streptozotocin-induction of diabetes produced changes in the neurotransmitter content of brain stem along with a significantly elevated blood glucose. The NE content of brain stem remained unchanged, but EPI content increased significantly. The turnover rate for NE to EPI that calculated for the diabetic stem showed a significant increase. Previous studies have shown that the epinephrine and its regulation in adrenergic nerve ending were controlled by alpha₂ adrenergic receptors (13,25,26). So the above observed changes in neurotransmitter content might be indicating an involvement of epinephrine mediated receptor alterations. This prompted us to study the alpha₂ adrenergic receptor kinetics. The Scatchard analyses using [³H] yohimbine showed that alpha₂ adrenergic receptors in diabetic brain stem have a significantly reduced affinity for the ligand. The

Table III

Binding parameters from clonidine inhibited [³H]NE binding in brain stem of diabetic rats

Animal Status	Best fit model	log (EC ₅₀)-1	log (EC ₅₀)-2	Ki-1(M)	Ki-2(M)
Control	Two-site	-9.798	-3.686	1.749 x 10 ⁻¹⁴	2.266 x 10 ⁻⁶
Diabetic	Two-site	-9.879	-3.408	2.301 x 10 ⁻¹⁴	6.806 x 10 ⁻⁶
Diabetic + Insulin	Two-site	-9.997	-3.506	1.978 x 10 ⁻¹⁴	2.976 x 10 ⁻⁶

Data are from the curves as determined from nonlinear regression analysis using computer program PRISM (see methods) and a one-site or two-site model. The affinity for the first and second site for the competing drug is designated as Ki-1 (for high affinity) and Ki-2 (for low affinity). EC₅₀ is the concentration of competitor that competes for half the specific binding and it is same as IC₅₀. The equation built-in to the program is defined in terms of the log (EC₅₀). It is argued that if the concentrations of unlabelled compound are equally spaced on a log scale, the uncertainty of the log (EC₅₀) will be symmetrical, but uncertainty of the EC₅₀ will not be symmetrical

Table IV

Binding parameters from drug inhibition studies of epinephrine inhibited [³H]yohimbine binding in control brain stem

Drug	Best-fit model	log(EC ₅₀)-1	log(EC ₅₀)-2	Ki-1(M)	Ki-2(M)	Hill slopes
Epinephrine(EPI)	Two-site	-7.647	-4.655	4.690 x 10 ⁻⁸	4.604 x 10 ⁻⁶	0.3026
EPI+ Gpp[NH]p	One-site	Nil	-5.292	Nil	1.064 x 10 ⁻⁶	0.7006
EPI + NaCl	One-site	Nil	-5.214	Nil	1.042 x 10 ⁻³	0.8704
EPI + Gpp[NH]p + NaCl	One-site	Nil	-6.686	Nil	4.291 x 10 ⁻⁸	0.8533

Data are from the displacement curves as determined by nonlinear regression analysis using the computer program PRISM (see methods) and a one-site Vs two-site binding model. The GTP and sodium ions render the receptor affinity into a single affinity.

receptors exist in two populations; one with a high affinity (Ki-1) and another with a low affinity (Ki-2). In diabetic state the ligand binding was weaker for the low affinity sites. Insulin treatment reversed these changes to normal. An immediate consequence of this affinity changes could be an increase in the receptor number of the subspecies of low affinity receptors. In our hands by using [³H] yohimbine as the ligand we were not able to demonstrate the two affinity states by Scatchard analysis. This limits our ability to show upregulation of this subtype of receptors. From the present analysis it could only be argued that in diabetic BS alpha₂ adrenergic receptors identified by clonidine seem to have reduced affinity for the Ki-2 sites, which may result in a consequent increase in the population of receptors.

Table V

Binding parameters from drug inhibition studies of epinephrine inhibited [³H]yohimbine binding in diabetic brain stem

Drug	Best-fit model	log(EC ₅₀)-1	log(EC ₅₀)-2	Ki-1(M)	Ki-2(M)	Hill slope
Epinephrine(EPI)	Two-site	-4.737	-7.142	5.080 x 10 ⁻⁸	1.997 x 10 ⁻⁶	0.70
EPI+ Gpp[NH]p	Two-site	-6.731	-4.627	5.690 x 10 ⁻⁸	7.215 x 10 ⁻⁶	0.62
EPI + NaCl	One-site	Nil	-0.9010	Nil	3.853 x 10 ⁻²	1.00
EPI + Gpp[NH]p + NaCl	One-site	Nil	-5.420	Nil	1.168 x 10 ⁻⁶	0.85

*p<0.05 compared to control

Data are from the displacement curves as determined by nonlinear regression analysis using the computer program PRISM (see methods) and a one-site Vs two-site binding model. The GTP and sodium ions render the receptor affinity into a single affinity.

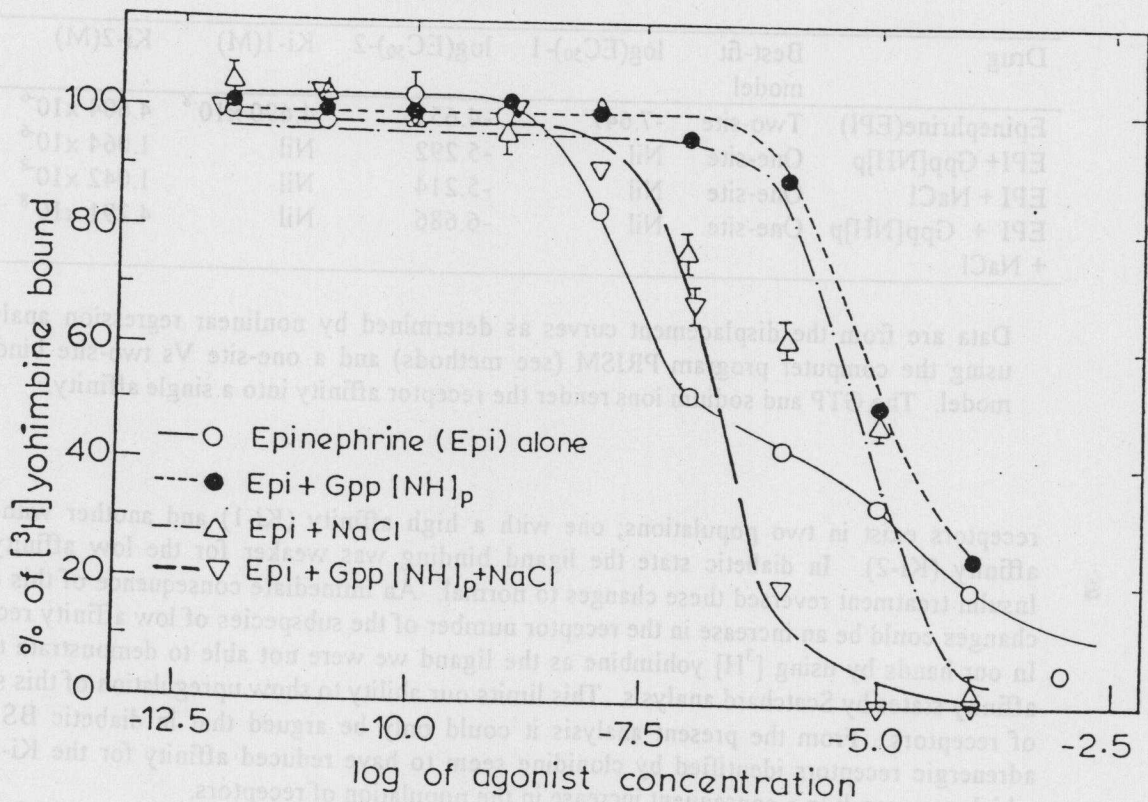


Fig. 1

Drug inhibition of specific [³H]yohimbine binding in control Brain stem. Each data point represents the mean \pm S.E.M. of six experiments, using 10nM of [³H]yohimbine. The curves are theoretical plots derived from nonlinear regression analysis based on a one-site Vs two-site model.

Diabetes associated CNS abnormality was characterized by progressive alterations of neurotransmitters and of transduction Gi / Go proteins (29). A quantitative analysis of these proteins in STZ model showed that there were no gross changes in either content of G proteins or m-RNA level. In a recent study attenuation of clonidine induced anti-nociceptive effect was suggested to be due to a central α_2 adrenoceptor desensitization and / or biochemical defect in the post receptor events in STZ diabetic rats (30). An altered G protein regulated affinity regulation observed in our study may show that these modifications of G proteins are either in its physiological status affecting the coupling with membrane effector systems or its structure.

The effects of insulin on adrenergic receptors indicate that the changes observed in adrenergic nerves are responding to insulin treatment. Even though there was no change in NE in STZ diabetic rats, insulin treatment to these rats decreased the NE content compared to the controls. As a result of this change, α_2 adrenergic receptors of D+I rats showed an elevated B_{max} and a reversion of affinity changes to control level, which might be a compensatory effect. From these results it is shown that insulin is able to correct the blood glucose level and some of the metabolic disturbances to that of control, there are changes in the metabolic enzymes (31) and neurotransmitter receptors which are altered by its treatment.

The serotonergic activity of diabetic brain stem showed an increase that is reversed by insulin treatment. The diabetic brain 5-HT, 5-HTP and 5-HIAA contents, when compared to control, showed that an accumulation of 5-HT may be due to a disturbance both in the synthesis as well as break-down pathways. Insulin-treated rats tried to correct the synthetic pathway with the result of a decrease in 5-HTP. The insulin was found to correct the altered 5-HT pathway by decreasing the turn over rate of 5-HTP to 5-HT. Our results on serotonin content are not consistent with an earlier report of decrease in 5-HT (7). The disparity in the data with this report could be related to difference in the methodology.

The serotonin receptor kinetics showed that the value of K_d increased in diabetic rats. This suggested that serotonin receptors have a decreased affinity identified using high affinity concentrations of the ligand (18). The earlier reports of 5-HT_{1A} and 5-HT₂ receptor kinetics in brain stem of diabetic rats showed that the receptor number of specific receptor subclasses increased significantly (7). Our observations showed a decreased affinity in the [³H]5-HT binding to the high affinity serotonin receptors. The changes observed in kinetic parameters identified by the specific ligands to the specific receptors might be important (7). When natural ligand is used for the high affinity serotonin receptors, the total added effect might be different. The collective effect in receptor kinetics is shown in our observation. Insulin treatment reversed the altered receptor affinity to normal. A reversal is evident in neurotransmitter and its metabolites.

In conclusion, in diabetic brain stem the noradrenergic and serotonergic activities were altered. These changes were associated with a changed post-receptor mechanism of α_2 adrenergic receptors and a decreased functional high affinity serotonergic receptor. The insulin treatment reversed these altered parameters to control level. Thus, it is suggested that the changes observed in the present study may be directly related to pancreatic insulin secretion and its functional inter-relationship with brain neurotransmitter receptors.

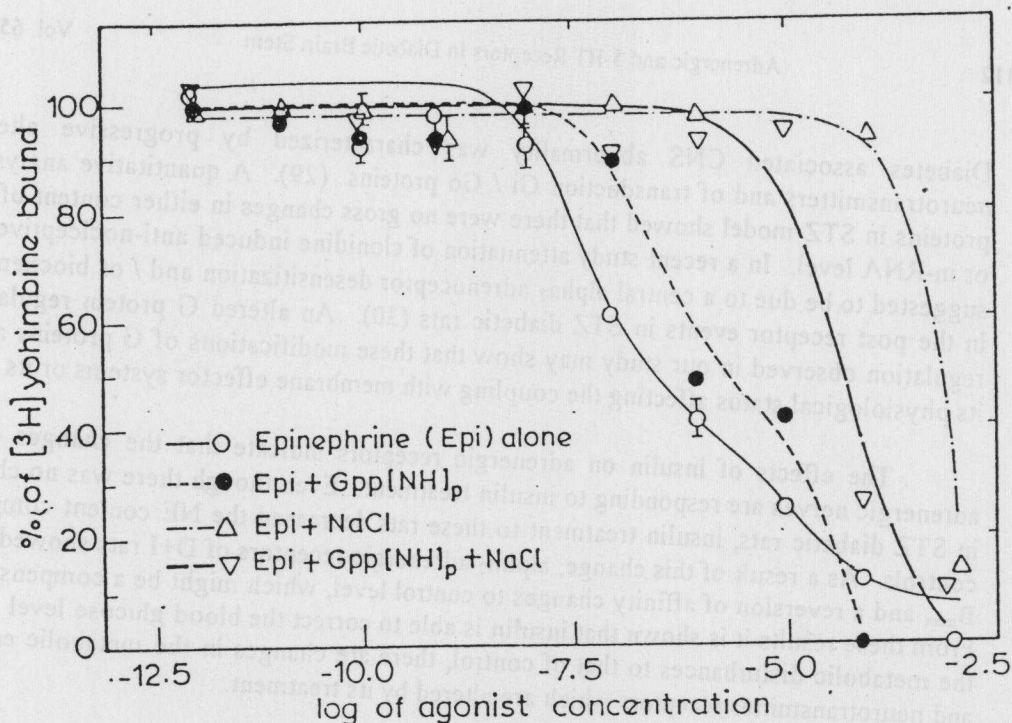


Fig.2

Drug inhibition of specific [³H]yohimbine binding in diabetic Brain stem. Each data point represents the mean \pm S.E.M. of six experiments, using 10nM of [³H]yohimbine.

The affinity changes of the α_2 adrenergic receptors were shown to be regulated by G proteins and sodium ion levels (16). Our analysis on the affinity states of these receptors by displacement studies of [³H]yohimbine against (-)-epinephrine showed that in control rats the α_2 adrenergic receptors in BS occur in two different affinity states. The GTP analogue when added shifted these receptors to a single affinity state in control animals. In diabetic animals also the α_2 adrenergic receptors in BS occur in two affinity. The K_i values for lower affinity states are different in control and diabetic BS when different competing drugs were used in our analysis (Tables III, IV and V). A difference in the K_i values for sites identified by yohimbine and clonidine have been reported (27). The observed difference in value of K_i -2 in diabetic BS compared to control when these two different pharmacological agents were used could be due to their difference in the ability in identifying various subtypes of α_2 -adrenoceptors. In diabetic BS, when GTP analogue added, it failed to shift the curve to a single lower affinity state. The curve fitted for a model for two-site binding, and the affinity for the K_i -2 decreased significantly compared to control and its K_i -1 values. These results showed that GTP analogue failed to shift the affinity states in diabetic rats. The regulatory effect on affinity of adrenergic receptors demonstrated using monovalent cations was intact in control and diabetic rats. When both compounds added together the shift to a single affinity state was evident in control and diabetic rats. In control rats when sodium ions and Gpp[NH]_p added the affinity for K_i -2 increased; whereas in diabetic rats the value of K_i -2 increased which indicate a decreased affinity compared to control, yet a shift to one single lower affinity is evident. This may be indicating an involvement of G-protein component in the sodium ion mediated regulations for adrenoceptors. From these results it is conceivable that in diabetic rats there is an altered post-receptor function at the G-protein affinity regulations. In a similar study to analyze the activation of phosphoinositidase through G protein coupled receptors in STZ-diabetic rats it is proposed that in peripheral nerves G protein function in cell signaling is impaired (28).

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