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Diabetes attenuates the response of the lumbospinal noradrenergic system to idazoxan

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Abstract

Allodynia is a common feature of painful diabetic neuropathy. This phenomenon appears to be under endogenous noradrenergic control and can be ameliorated effectively by α_2-adrenoceptor agonists. Accordingly, diabetic lumbospinal noradrenergic dynamics was evaluated using high performance liquid chromatography with electrochemical detector (HPLC-ECD), in vitro ligand binding and RT–PCR-based techniques. Streptozotocin (STZ)-treated and Goto–Kakizaki (GK) diabetic rats were included, respectively, as models for type I (insulin-dependent) and type II (non-insulin-dependent) diabetes mellitus. The data from these studies revealed that lumbospinal norepinephrine (NE) release, as indicated by the 3-methoxy-4-hydroxyphenyl glycol (MHPG)/NE ratio, was decreased as a function of diabetes. Similarly, the binding density of [^{3}H] *p*-aminoclonidine and the level of expression of mRNA transcripts encoding for the α_{2A}-adrenoceptor subtype and noradrenergic transporter were also reduced in this disease state. Analogous findings were obtained in non-diabetic Wistar rats rendered hypercortisolemic by the subcutaneous implantation of slow releasing pellets containing a supraphysiological dose of glucocorticoid (GC). Tactile allodynia was consistently observed in STZ- and GC-treated animals. The responsiveness of α_2-adrenoceptors to idazoxan (α_2-adrenoceptor antagonist) indicated a dose-dependent enhancement of noradrenergic transmission in lumbar segments of normal spinal cord. In stark contrast, this neurochemical action of idazoxan was attenuated in diabetic and hypercortisolemic animals. The institution of insulin therapy ameliorated diabetes-related abnormalities in lumbospinal noradrenergic dynamics. Overall, the current finding suggests that diabetic and hypercortisolemic allodynic symptoms may stem from, at least in part, down-regulation of α_2-adrenoceptors in these disease states.

Keywords: α_2-Adrenoceptor; Idazoxan; Diabetes mellitus; Spinal cord; Hypercortisolemia; Allodynia

1. Introduction

Dysfunction of sensory motor and autonomic neurons is a consistent feature of long-term diabetic complications [28]. Muscle atrophy and weakness, decreased motor ability, impotence, allodynia and hyperalgesia [in response to both non-noxious (thermal and mechanical) and noxious (thermal, mechanical and chemical stimuli) are problems often encountered during the course of diabetes [16,28,58,63,65]. A common anatomical site location for these diabetes-related deficits is the spinal cord, since it represents the main highway for the conduction of peripheral information and central commands. In addition, the spinal cord is involved in integrating nociceptive, proprioceptive as well as mechanoreceptive functions. While information regarding spinal cord function in diabetes is somewhat limited, a number of studies have reported a reduction in axonal content of neurofilaments and micro-tubules, loss of synapses, dwindling of axonal diameters, a decrease in axonal transport and an impairment of nerve conduction velocity [2,13,16,24,46].

Bulbospinal monoaminergic descending inhibitory pathways emanating from the parabrachial nuclei, locus coeruleus (norepinephrine, NE) and medullary–pontine raphe nuclei (serotonin) modulate pain transmission within the spinal cord [8,26,45,67,68]. For example, stimulation of discrete sites in the brain promotes spinal release of NE with

Abbreviation: STZ, streptozotocin; GC, glucocorticoid; NAT, noradrenergic transporters; RT–PCR, reverse-transcriptase polymerase chain reaction; HPLC-ED, high performance liquid chromatography with electrochemical detector; MHPG, 3-methoxy-4-hydroxyphenyl glycol; NE, norepinephrine; GK, Goto–Kakizaki.

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consequent blockade of spinal nociceptive reflexes [30]. This analgesic action is thought to be mediated by NE-related activation of dorsal horn α_2-adrenoceptors [39,61], of which four subtypes have recently been identified α_{2A}, α_{2B}, α_{2C} and α_{2D} [40,52]. The α_{2D}-adrenoceptor subtype was determined to be a rat homologue of the human α_{2A}-adrenoceptor. Indeed systemic and intrathecal administration of α_2-adrenoceptor agonists including clonidine and dexmedetomidine have been shown to elevate nociceptive thresholds both in human and animal models of pain [18,41–43].

The pivotal role of NE in modulating pain transmission was further corroborated by a previous report indicating that the mechanical and thermal allodynia observed in the spinal nerve ligation model of neuropathic pain are under endogenous, noradrenergic control [69]. In this connection, both tricyclic antidepressants, inhibitors of NE reuptake and the α_2-adrenergic agonists have been shown to be effective in attenuating the associated neuropathic pain of ligated nerves and diabetes [23,70,71]. In view of the information summarized above and in consideration of numerous experimental and clinical data documenting that the diabetic state is associated with both thermal and tactile allodynia [16], as well as a deficit in central noradrenergic function [4,6], a hypothesis was formulated stating that certain features of diabetic neuropathic pain may involve an aberration in lumbospinal α_2-adrenoceptors. As a first step toward garnering relevant data regarding the above proposal, we conducted a neurochemical study of the lumbospinal noradrenergic system during diabetes. In addition, the response of this system to the selective α_2-adrenoceptor antagonist idazoxan was also addressed. Streptozotocin (STZ)-treated Wistar and Goto–Kakizaki (GK) rats were used, respectively, as models of type I (insulin-dependent) and type II (non-insulin-dependent) diabetes mellitus.

2. Materials and methods

2.1. Animals

GK spontaneously diabetic and age-matched Wistar rats (Kuwait University breeding colony) were housed on a 12-h light–dark cycle (lights on from 0600 h). The ambient temperature was kept at 22°C and the rats had free access to standard laboratory food and tap water. At all times the rats were handled in accordance with the ethical guidelines for investigations of experimental pain in conscious animals [72].

2.2. Diabetes induction, insulin and steroid treatment

Diabetes was induced in male Wistar rats by an intravenous (i.v.) injection of STZ (55 mg/kg b.wt) dissolved in 0.05 M sodium citrate buffer pH 4.5; control rats received buffer alone by the same route. Diabetic animals were randomly subdivided into two groups, with one group receiving no antidiabetic treatment while the other was subjected to daily subcutaneous injection of 5–8 U of a mixture of crystalline zinc insulin/lente insulin at a ratio of 1:3. The insulin regimen was started 3 days after the STZ injection and continued throughout the experimental period. The dose of insulin was determined on the basis of the daily urine and weekly blood tests of glucose. An additional group of non-diabetic Wistar rats were rendered hypercortisolemic by the subcutaneous implantation of slow-releasing pellets containing glucocorticoid (GC). The pellet was calibrated to release GC at a rate of 6.7 mg/day for 30 days. This supraphysiological dose of GC has been shown in our laboratory to reduce thymus tissue weight and increase hepatic activity of tryptophan pyrrolase, a heme-containing enzyme that is inducible by ethanol and GCs [27,33].

2.3. Assessment of noradrenergic dynamics

2.3.1. Tissue preparation

Animals derived from various experimental groups were fasted for 6 h before sacrifice by decapitation. Blood was collected into heparinized tubes, centrifuged at 3000 × *g* for 15 min and the plasma used for the measurement of insulin and glucose concentrations. Spinal cord was obtained by transecting the vertebral column at the level of the caudal equina and applying hydraulic pressure to the caudal opening of the spinal cord column with a saline-filled 20-ml syringe connected to an 18-gauge needle. Following removal, the spinal cord was chilled on an ice-cold glass plate. Two slices of the middle portion of the lumbar enlargement containing segments L_5 and L_6 were used for catecholamine analysis and RNA isolation. The rest of the spinal cord was used for the assay of ligand binding in vitro. The above measurements were also conducted on other areas of the central nervous system (CNS) including hypothalamus, hippocampus and brain stem.

2.3.2. Drug treatment and NE metabolism

Several doses of idazoxan were evaluated in preliminary experiments based on previously published data [37]. The selected doses are in the range of effective concentrations under the threshold to produce behavioral side effects. Animals derived from various experimental groups were injected i.p. with doses of idazoxan, dissolved in physiological saline (2.5, 5, 10 and 20 mg/kg b.wt). Sixty minutes later, animals were sacrificed and spinal cord and brain were processed as described above.

Measurement of NE and 3-methoxy-4-hydroxypheny glycol (MHPG) concentrations in discrete areas of the brain and spinal cord was performed by high pressure liquid chromatography with electrochemical detection (HPLC-ECD, BAS 200B, West Lafayette, IN) [5]. On the day of the assay, samples were thawed and sonicated (550 Sonic Dismembrator, Fischer Scientific) in 10 volumes (w/v) of

0.1 M sodium acetate buffer pH 5.0 containing 5 U/ml ascorbic acid oxidase and 20 ng/ml of dihydroxybenzylamine (DHB) as an internal standard. This enzymatic addition markedly reduced the front peak, thus making the first peak representing MHPG more readily detectable. An aliquot (100 μl) of the tissue homogenate was incubated with 25 μl of sulfatase (15 mg/ml, Sigma, St. Louis, MA) at 37°C for 2 h, in order to liberate conjugated MHPG into its free form so that it could be detected under our chromatographic conditions. The reaction was terminated by the addition of enough perchloric acid to establish a final concentration of 0.05 M. To maximize protein precipitation, samples were incubated overnight at 4°C and then centrifuged at 15000 × *g* for 20 min. The resulting supernatants were filtered and 20 μl was injected onto a C-18 reverse-phase column (Waters, Nova-Pak, 150 × 3.9 mm, 4 μm spheres). The pellets were dissolved in 0.5 M NaOH and assayed for protein. The HPLC mobile phase consisted of a mixture of 0.02 M sodium citrate, 0.01 M sodium phosphate, 0.003 M octanesulfonic acid, 0.003 M hexanesulfonic acid and 0.15 mM EDTA. A total of 5 ml of *o*-phosphoric acid (85%) and 3.0 ml of diethylamine were added per liter of solution. The final isocratic solvent was a combination of 98.4% of the former solution, 1.2% acetonitrile and 0.4% methanol at a pH of 3.1. This mobile phase was filtered and degassed using a solvent filtration apparatus with GS 0.22 μm (pore size) Millipore filters (Waters, Milford, MA). Subsequently, it was sparged with helium continuously and recirculated through the HPLC system. The EC detector was a LC-4B electronic controller (BAS) with a dual glassy carbon working electrode. The applied potential was +0.75 V versus an Ag/AgCl reference electrode with the sensitivity set at 1 and 2 nA. NE and MHPG concentrations were established from the peak area ratio using an internal/authentic standard and the external program (Chromgraph Report 4.0). Retention times in minutes were: MHPG, 1.95; NE, 2.33.

2.3.3. Receptor binding

Spinal cord tissue was thawed and homogenized in ice cold 0.32 M sucrose–0.05 M Tris pH 7.4 (1:10 w/v) and centrifuged at 1000×*g* for 10 min. The supernatant was removed, stored on ice and the resulting pellet was re-homogenized in Tris buffer and centrifuged at 1000 × *g* for 10 min. The synaptosomes remaining in the combined supernatant were pelleted by a subsequent centrifugation at 30,000×*g* for 20 min. The pellet was washed in 10 volumes of fresh buffer (50 mM Tris–HCl buffer pH 7.4 containing 1mM $MgCl_2$) and recentrifuged. The final pellet was resuspended in the same buffer (equivalent to 15 mg wet weight of tissue/ml) and used immediately in the binding assays [32]. All centrifugations were carried out at 4°C. [^{3}H]*p*-aminoclonidine was added to the synaptosomal preparation, which contained 300–400 μg protein in 1 ml binding buffer (50 mM Tris–HCl pH 7.4 and 1 mM $MgCl_2$) to provide a final concentration of 0.1–5 nM [^{3}H]*p*-aminoclonidine. The binding was allowed to reach equilibrium at 22°C for 45 min. Nonspecific binding was determined using 10 μM phentolamine. The membranes were separated by filtration with a Whatman GF/B filter and washed twice with 5 ml of ice-cold buffer (50 mM Tris–HCl pH 7.4 and 1 mM $MgCl_2$). Radioactivity bound to the membrane was determined by liquid scintillation counting using Beckman ready made scintillation cocktail, with an efficiency of 45%.

2.3.4. RNA isolation and reverse transcriptase–polymerase chain reaction (RT–PCR)

Total cellular RNA was isolated from sections of spinal cord lumbar enlargement containing segments L_5 and L_6 using the acid guanidinium isothiocyanate–phenol-chloroform method of Chomczyaski and Sacchi [15]. Briefly, tissue (100 mg) was homogenized in 1 ml of denaturing solution consisting of 4 M guanidinium isothiocyanate, 0.25 M sodium citrate pH 7.0, 0.5% sarcosyl and 0.1 M 2-mercaptoethanol. RNA was extracted with a mixture of 3 M sodium acetate pH 4.5, phenol and chloroform-isoamyl alcohol. 49:9. The RNA contained in the aqueous layer was precipitated with 70% ethanol and the final RNA pellet dissolved in water. The optical density at 260 nm was determined to estimate the amount of total RNA before it was used in RT–PCR.

DNase-treated RNA from the lumbar enlargement of the spinal cord was used as the template for the first strand cDNA synthesis. Aliquots (4 μg) of total RNA were annealed with 1 mM of oligo d(T) 16 primer (Perkin Elmer, Madison, WI) by heating for 10 min at 70°C followed by slow cooling to 37°C. RT reactions were carried out in a total volume of 20 μl containing oligo dT-annealed RNA, 50 mM Tris–HCl, 75 mM KCl, 3.0 mM $MgCl_2$, 10 mM DTT, 1 mM each of dATP, dCTP, dGTP and dTTP (Pharmacia, Sweden), 20 units RNA guard (Pharmacia) and 25 units Avian myeloblastosis virus reverse transcriptase (Pharmacia). Extension was initiated at 42°C for 60 min and then terminated at 80°C for 10 min.

PCR reactions were conducted in a total volume of 20 μl containing 4 μl of the RT reaction product, 2 μl 10 × PCR buffer (100 mM Tris–HCl, pH 8.3, 500 mM KCl), 1.5 mM $MgCl_2$, 0.2 mM NTPs, 1 unit DNA polymerase (Ampli Taq, Perkin Elmer Cetus) and 50 pmol primer set specific for α_{2A}-adrenoceptor (forward primer, 5′-CGT GCT GGT TAT TAT CGC AGT-3′; reverse primer, 5′-CCC TTC TTC TCT ATG GAG ATG-3′) or noradrenergic transporter (NAT, forward primer, 5-GCT TCT ACT ACA ACG TCA TCA TC-3; reverse primer 5-CGA TGA CGA CGA CCA TCA G-3). The oligonucleotide primers used to generate a 333-bp (α_{2A}-adrenoceptor) or 294 bp (NAT) fragment were described previously [34,36].

The PCR amplification step was carried out using a programmable Perkin Elmer Gene-Amp PCR-960. The thermal profile includes denaturing at 94°C for 1 min, (α_2-adrenoceptor, histone, NAT) annealing at 57°C for 1

min (α_2-adrenoceptor, histone) or 2 min (NAT) and extending at 72°C for 1 min (α_2-adrenoceptor, histone) or 2 min (NAT). As a final extension step, the reaction was heated for 7 min at 72°C. Relative differences in gene expression were determined after optimizing the following PCR conditions: (1) The number of cycles for each primer set was carefully selected such that the PCR products were within their linear range (α_{2A}-adrenoceptor, 32 cycles; NAT, 40 cycles; histone, 22 cycles). 2) At the selected cycle number, different amounts of total RNA were added to ensure that the amplification of the specific PCR products were linear with respect to input RNA. Negative controls included the omission of RNA or reverse transcriptase. The PCR products were separated on ethidium bromide stained 2% agarose gels and photographed by a gel documentation system (BioMetra, Germany). Negatives were scanned on a densitometer to obtain the area of the bands using ScanPacK 3.0 gel analysis software (BioMetra). The results are reported in relative densitometric units (arbitrary) in the figures. Diabetes appears not to have any significant effect on the house keeping gene histone. Thus, histone mRNA was used as an internal standard to minimize the possible sample to sample variability in the total cellular RNA applied to the ethidium bromide stained agarose gel. The level of α_{2A}-adrenoceptor or NAT mRNA in the rat spinal cord of different treatments is expressed as the ratio of α_{2A}-adrenoceptor or NAT mRNA to histone mRNA. Triplicate quantification of the same total RNA sample from different treatment groups showed a variability of 5–10% (mean 5.5%; $n=8$ per treatment group).

2.3.5. Pain test

Assessment of mechanical allodynia was achieved by subjecting the dorsal surface of the animals' hind paw to pressure from a von Frey monofilament. Rat vocalization or paw withdrawal was recorded as a positive response.

2.3.6. Plasma glucose and insulin

Plasma glucose levels were measured with the *o*-toluidine method [25]. Immunoreactive insulin was quantified by radioimmunoassay using polyethylene glycol method with rat insulin as a standard [21].

2.3.7. Protein

Tissue protein contents were determined as described previously using bovine serum albumin as a standard [38].

2.4. Data analysis

The kinetic parameters (e.g. K_d and B_{max}) of the saturation binding data were analyzed using the Eadie–Hofstee plot. Data on the effects of diabetes on spinal cord contents of catecholamines, adrenoceptor binding and the abundance of mRNA transcripts encoding for α_{2A}-adrenoceptor and NAT were analyzed by one-way or two-way ANOVA followed by Tukey-Krmer's multiple comparison test. The results of all statistical tests were considered significant if $p < 0.05$. All values are reported as means ± S.E.M.

3. Results

Physical and biochemical data revealed that the gain in body weight of control Wistar and GK diabetic rats was, respectively, about 28 and 26 g per week for a total duration of 4 to 5 weeks. In contrast, the rate of growth was markedly reduced in Wistar STZ-diabetic (− 5.8 g/week) and hypercortisolemic (− 10 g/week) animals. Plasma glucose levels (mg/100 ml) were elevated in both STZ (STZ-D 475 ± 33 vs. control 133 ± 8) and GK (GK-D 326 ± 20 vs. control 133 ± 8) diabetic rats. Hypercortisolemic Wistar rats appeared to be normoglycemic. The plasma insulin level (ng/ml) was not statistically different between the GK-D and the corresponding control values (GK-D 1.29 ± 0.18 vs. control 1.03 ± 0.14). A marked decrease (0.32 ± 0.07) in the level of this hormone was evident, however, in the STZ-D rats. Both diabetic and hypercortisolemic animals consistently demonstrated tactile allodynia as reflected by the response threshold to pressure exerted by a von Frey monofilament (Fig. 1). Control animals tested concurrently rarely showed any response to the von Frey filament below the cut off of 80 g.

Diabetic and hypercortisolemic states appear to alter several parameters of CNS noradrenergic dynamics. These include (i) rate of NE release as reflected by the MHPG/NE ratio, (ii) saturation isotherm of [^{3}H]*p*-aminoclonidine

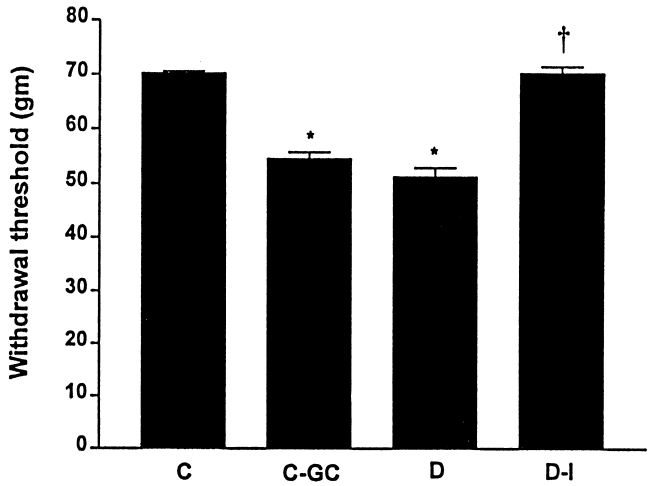

Fig. 1. Response threshold to von Frey filament stimulation of the hindpaw of control and diabetic rats receiving placebo or drug treatment. Induction of diabetes and hypercortisolemic state together with insulin treatment were conducted as described in the legend of Table 1. Values are the means ± S.E.M. of at least eight animals/group. Abbreviations: Cont, control; C-GC, glucocorticoid-treated control; STZ-D, streptozotocin-treated diabetic; STZ-DI, insulin-treated diabetic; GK-D, Goto–Kakizaki diabetic. * Significantly different from corresponding control values at $p < 0.05$; † significantly different from corresponding vehicle-treated diabetic at $p < 0.05$.

binding to spinal cord synaptosomal preparation and (iii) levels of expression of mRNA transcripts encoding for NAT and α_2-adrenoceptor. NE contents in lumbar segments of the spinal cord of STZ-D and GK-D rats were increased by 17% and 32%, respectively, over their corresponding control values (Table 1). In stark contrast, a decrease in MHPG level STZ-D (30%) and GK-D (35%) was evident in these animals Table 1. The specific binding of various concentrations of [^{3}H]*p*-aminoclonidine (0.1–5 nM) to spinal cord synaptosomal preparations appears to be a saturable process. Eadie–Hofstee analysis of equilibrium isotherms revealed a single class of binding sites with high affinity and low capacity. Maximum binding (B_{max}) of [^{3}H]*p*-aminoclonidine to synaptosomal preparations from STZ-D and GK-D rats was decreased, by 38% and 36%, respectively, as compared to corresponding control values (Table 2). However, the dissociation constant K_d, was elevated but only in the GK-D rats (Table 2).

The α_{2A}-adrenoceptor subtype has been reported to predominate in the spinal cord and also play a role in the modulation of nociceptive impulses [35,44,49,53,59]. Thus, it was measured in diabetes, together with NAT, using the highly sensitive RT–PCR assay. Optical density-based quantification of multiple PCR blots revealed that the expression of lumbospinal mRNA transcripts encoding for α_{2A}-adrenoceptor and NAT was decreased as a function of diabetes (Fig. 2). Although there are some pitfalls associated with the RT–PCR assay, the conclusions derived from the present experiments are supported by a number of controls. (i) Under optimal conditions, only one PCR band was visible on gels. (ii) The RNA samples used in the RT–PCR reactions were free of carry-over or genomic DNA contamination. For example, amplification of the negative controls containing no reverse transcriptase or RNA revealed no detectable PCR products. Most of the aforementioned neurochemical parameters in the STZ-D rats were partially restored towards normal values following the institution of insulin therapy (Tables 1 and 2 and Figs. 1 and 2).

Table 1
Lumbo-spinal NE metabolism in diabetic and hypercortisolemic states

Treatment	MHPG (pmol/mg protein)	NE (pmol/mg protein)	MHPG/NE
Cont	3.22 ± 0.03	18.81 ± 0.24	0.17 ± 0.001
C-GC	2.73 ± 0.05*	21.30 ± 0.24*	0.13 ± 0.006*
STZ-D	2.68 ± 0.03*	22.02 ± 0.18*	0.12 ± 0.004*
STZ-DI	2.90 ± 0.05	18.45 ± 0.18†	0.16 ± 0.001†
GK-D	2.72 ± 0.04*	24.91 ± 0.52*	0.11 ± 0.003*

Diabetic and hypercortisolemic states in Wistar rats were induced, respectively, by a single injection of STZ (55 mg/kg, i.v.) and the subcutaneous implantation of slow releasing pellet containing GC (6.7 mg/day for 30 days). Insulin therapy consisted of daily subcutaneous injection of 5–8 U of regular and lente insulin (1:3). HPLC-ECD based technique was used to determine lumbospinal NE metabolism. All determinations were done in duplicate, values are expressed as means ± S.E.M. for at least seven animals per treatment group. Abbreviations: Cont, control; C-GC, glucocorticoid-treated control; STZ-D, streptozotocin-diabetic; STZ-DI, insulin-treated diabetic; GK-D, Goto–Kakizaki diabetic.

* Significantly different from corresponding control values at $p < 0.05$.

† Significantly different from corresponding vehicle-treated diabetic at $p < 0.05$.

Table 2
Spinal cord α_2-adrenoceptor binding in diabetic and hypercortisolemic states

Treatment	[^{3}H]*p*-aminoclonidine	
	B_{max} (fmol/mg protein)	K_d (nM)
Cont	66.90 ± 5.3	2.85 ± 0.31
C-GC	47.53 ± 7.6*	2.69 ± 0.28
STZ-D	41.75 ± 4.7*	2.73 ± 0.22
STZ-DI	58.66 ± 8.1†	2.97 ± 0.44
GK-D	43.10 ± 3.0*	0.63 ± 0.29*

Induction of diabetes and hypercortisolemic states as well as insulin treatment were conducted as described in the legend of Table 1. Dissociation constant (K_d) and maximum binding capacity (B_{max}) were determined by Eadie–Hofstee analysis of specific [^{3}H]*p*-aminoclonidine binding. Values are expressed as means ± S.E.M. from triplicates of at least seven animals per group. Abbreviations: Cont, control; C-GC, glucocorticoid-treated control; STZ-D, streptozotocin-diabetic; STZ-DI, insulin-treated diabetic; GK-D, Goto–Kakizaki diabetic.

* Significantly different from corresponding control values at $p < 0.05$.

† Significantly different from corresponding vehicle-treated diabetic at $p < 0.05$.

Non-diabetic control animals rendered hypercortisolemic by the chronic administration of supraphysiological doses of GC exhibited neurochemical features with high degrees of resemblance to that seen with STZ-D and GK-D rats. For example, MHPG/NE ratio, B_{max} of *p*-aminoclonidine and mRNA transcripts encoding for α_{2A}-adrenoceptor subtype and NAT in hypercortisolemic animals were

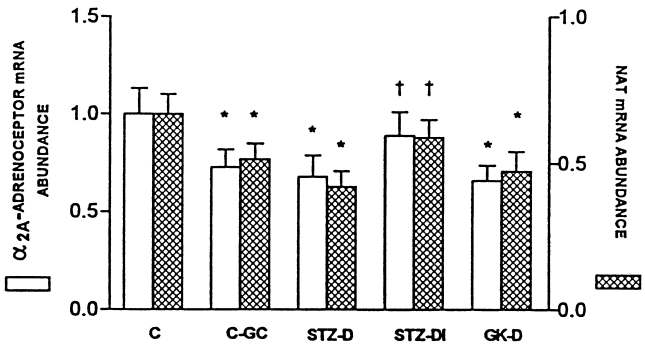

Fig. 2. Densitometric analysis of mRNA expression of lumbospinal α_{2A}-adrenoceptor and NAT in diabetic and hypercortisolemic states. Induction of diabetes and hypercortisolemic state together with insulin treatment were conducted as described in the legend of Table 1. Values are the mRNA levels relative to the house keeping gene histone. All were compared to the control value that was defined as 1.0. Each value is the mean ± S.E.M. for three different RNA preparation, of six animals each. Abbreviations: Cont, control; C-GC, glucocorticoid-treated control; STZ-D, streptozotocin-treated diabetic; STZ-DI, insulin-treated diabetic; GK-D, Goto–Kakizaki diabetic. * Significantly different from corresponding control values at $p < 0.05$; † significantly different from corresponding vehicle-treated diabetic at $p < 0.05$.

Table 3
Brain norepinephrine-metabolism in diabetic and hypercortisolemic states

	MHPG			NE			MHPG/NE		
Treatment	HYP (pmol/mg)	HIP (pmol/mg)	BST (pmol/mg)	HYP (pmol/mg)	HIP (pmol/mg)	BST (pmol/mg)	HYP	HIP	BST
Cont	19.4 ± 0.3	8.9 ± 0.4	6.7 ± 0.2	88.3 ± 5.0	34.9 ± 0.6	28.8 ± 0.5	0.22 ± 0.02	0.25 ± 0.01	0.23 ± 0.02
C-GC	16.4 ± 0.5*	6.4 ± 0.3*	5.4 ± 0.4*	97.0 ± 2.3	40.5 ± 0.6*	31.5 ± 0.9	0.17 ± 0.04*	0.16 ± 0.01*	0.17 ± 0.04*
STZ-D	16.0 ± 0.9*	6.5 ± 0.2*	5.4 ± 0.4*	108.8 ± 0.7*	50.1 ± 1.8*	34.3 ± 0.5*	0.15 ± 0.01*	0.13 ± 0.03*	0.16 ± 0.02*
STZ-DI	18.8 ± 1.2†	9.7 ± 0.3†	6.5 ± 0.3†	86.3 ± 5.4†	36.3 ± 0.8†	28.8 ± 0.3†	0.22 ± 0.01†	0.26 ± 0.04†	0.23 ± 0.01†
GK-D	14.50 ± 0.5*	7.3 ± 0.3*	ND	106.8 ± 4	44.1 ± 3.0*	ND	0.13 ± 0.01*	0.16 ± 0.01*	ND

Induction of diabetic and hypercortisolemic states together with insulin treatment were conducted as described in the legend of Table 1. Values are expresses means ± S.E.M. of eight animals/treatment. Abbreviations: HYP, hypothalamus; HIP, hippocampus; BST, brain stem; Cont, control; C-GC, glucocorticoid-treated control; STZ-D, streptozotocin-diabetic; STZ-DI, insulin-treated diabetic; GK-D, Goto–Kakizaki diabetic. ND, not determined.

* Significantly different from corresponding control value at $p < 0.05$.

† Significantly different from corresponding diabetic value at $p < 0.05$.

reduced by 24%, 29%, 27% and 23%, respectively, when compared to corresponding control values (Tables 1 and 2 and Fig. 2).

Hypoactivity of noradrenergic system in diabetic and hypercortisolemic states is not limited to the lumbar segment of the spinal cord but extends to other areas of the brain including the hypothalamus, hippocampus and brain stem. For example, the rate of release of NE as indicated by MHPG/NE ratios was reduced in the hypothalamus, hippocampus and brain stem of both animal models of diabetes mellitus (Table 3). Similarly, these neurochemical changes were also seen in non-diabetic Wistar rats rendered hypercortisolemic with supraphysiological doses of GC. As in the lumbar segment of spinal cord, insulin treatment partially restored STZ-induced changes in brain noradrenergic metabolism (Table 3).

The functional responsiveness of α_{2A}-adrenoceptors was examined in the context of idazoxan's ability to enhance NE release as indicated by the MHPG/NE ratio. Idazoxan (2.5–20 mg/kg, i.p.), 60 min post-injection, produced a dose-dependent increase in NE release in normal spinal cord. The minimum effective dose (lowest dose to produce a statistically significant effect) was 5 mg/kg (idazoxan 0.82 ± 0.12 vs. vehicle 0.60 ± 0.04, Fig. 3). A further elevation over saline-treated values in MHPG/NE ratio was evident with idazoxan at 10 mg/kg (58%) and 20 mg/kg (98%) (Fig. 3). In stark contrast, the idazoxan-induced enhancement of lumbospinal noradrenergic transmission was attenuated in diabetic and hypercortisolemic animals (Fig. 3). Indeed, over the dose range studied, only the highest dose (20 mg/kg) was effective in promoting NE release (Fig. 3).

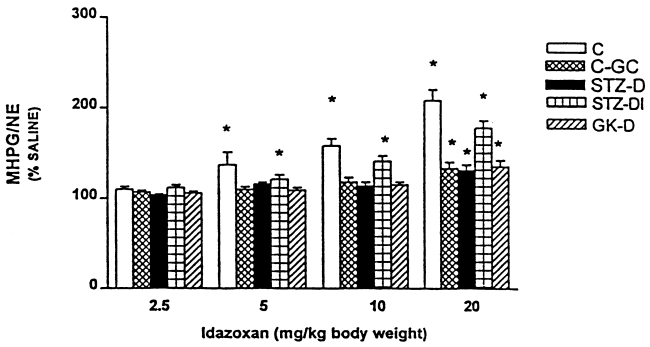

Fig. 3. Effect of idazoxan on lumbospinal MHPG/NE ratio in diabetic and hypercortisolemic states. Induction of diabetes and hypercortisolemic state together with insulin treatment were conducted as described in the legend of Table 1. Lumbospinal MHPG/NE ratio was determined 60 min after the administration of saline or various doses of idazoxan (2.5 mg to 20 mg/kg). Values are expressed as means ± S.E.M.; $n > 6$ per data point. Abbreviations: Cont, control; C-GC, glucocorticoid-treated control; STZ-D, streptozotocin-treated diabetic; STZ-DI, insulin treated diabetic; GK-D, Goto–Kakizaki diabetic. * Significantly different from corresponding saline-treated value at $p < 0.05$.

4. Discussion

This study investigates lumbospinal noradrenergic dynamics under diabetic and hypercortisolemic states. HPLC-ECD, in vitro radioligand receptor binding and RT–PCR-based techniques were used, respectively, to assess the rate of NE release, kinetic parameters (B_{max}, K_d) of α_2-adrenoreceptor functioning and the level of expression of mRNA transcripts encoding for NAT and the α_{2A}-adrenoreceptor subtype. The data derived from these studies indicate that lumbar segments of the spinal cord of both type I (STZ-treated Wistar rats) and type II (GK) animal models of diabetes mellitus were associated with several neurochemical abnormalities including: (i) an increase in tissue content of NE, (ii) a decrease in MHPG, a major metabolite of NE, (iii) a reduction in the MHPG/NE ratio, an index of NE turnover or release, (iv) a decrease in [^{3}H] *p*-aminoclonidine binding site density, and (v) a reduction in the level of expression of mRNA transcripts encoding for α_{2A}-adrenoreceptor and NAT. Most of these neurochemical changes also exist in STZ-treated Sprague–Dawley rats [4] and they were amenable to insulin therapy at doses that maintained near-normoglycemia. Together, these findings rendered unlikely the possibility that the abnormalities in CNS noradrenergic dynamics of Wistar and Sprague–Dawley rats stemmed from the toxic effects of STZ.

The above neurochemical data observed in GK rats, a new genetic model of non-insulin dependent diabetes, are novel and to the best of our knowledge has not been reported previously. No attempt was made in this study to delineate whether hyperglycemia, shared by both animal models of diabetes, hypoinsulinemia (STZ-D) or decreased insulin sensitivity (GK) contributed to the diabetes-related alterations in central noradrenergic dynamics.

Insulin- and non-insulin-dependent diabetes mellitus exemplified, respectively, by the STZ-Wistar and GK rats, appear to reduce the MHPG/NE ratio within the lumbar segment of the spinal cord as well as in other brain areas including hypothalamus, hippocampus and brain stem. The MHPG/NE ratio reflects to a large extent the rate of release of NE. In this context, an agonist such as clonidine, which suppresses central NE impulse flow, has been shown to be associated with a reduction in MHPG levels [9,54]. In contrast, an increase in noradrenergic neuronal activity produced by stress or the α_2-adrenoreceptor antagonists (e.g. yohimbine, idazoxan and piperoxan) resulted in a marked elevation of neuronal tissue content of MHPG [14,37,55,56,66]. Our data with Wistar STZ-D and GK-D rats compare favorably with those previously reported in the STZ Sprague–Dawley rats [4]. Together, these results are consistent with the concept that diabetes-related suppression of NE release within the CNS is neither strain- nor type-dependent.

Diabetes mellitus of both types appears to be associated with a reduction in central NE release or turnover as indicated by the MHPG/NE ratio. The cellular mechanism responsible for the development of this phenomenon is yet to be established. It has been reported, however, that the α_2-adrenergic autoreceptor modulates NE release in various tissues, including the CNS, by means of a negative feedback mechanism [60,64]. Indeed, α_2-adrenergic agonists such as clonidine and dexmedetomidine inhibited, whereas yohimbine and idazoxan enhanced, the release of NE from various areas of the CNS [17,20,62]. Accordingly, it is reasonable to suggest that diabetes-related suppression of noradrenergic neuronal transmission may stem from an up-regulation of the autoinhibitory α_2-adrenoceptor. Our current data do not support this proposition and clearly showed that binding sites and α_2-adrenoceptor mRNA levels are diminished during diabetes. A plausible explanation for this incongruity lies in the fact that diabetes may interfere with NE reuptake mechanism, thus provoking transmitter accumulation in the synapse and continued stimulation of α_2-adrenoceptors. Consequently, down-regulation of α_2-adrenoceptor would ensue concomitantly with a decrease in the rate of NE turnover. Indeed, the results regarding the lumbospinal density of α_2-adrenoceptors, MHPG/NE ratios and the levels of mRNA transcripts encoding for NAT and α_{2A}-adrenoceptors give credence to the above suggestion. Alternatively, synaptic degeneration in diabetic spinal cord may contribute, at least in part, to the above neurochemical abnormalities including a decrease in the maximum binding of α_2-adrenoceptors and rate of release of NE. This view is of interest in the light of previous studies showing that the spinal cord of diabetics is accompanied by loss of synapses and a reduction in axonal content of neurofilaments and microtubules [2,13,16,24,46].

The functional responsiveness of lumbospinal α_2-adrenoceptors in diabetes was evaluated in the context of idazoxan. This α_2-adrenoceptor antagonist facilitates the release of NE via the blockade of both somatodendritic and terminal autoreceptors [22,57]. Idazoxan has a considerable selectivity for the α_2-adrenoceptor, with an affinity ratio of about 30 for presynaptic α_2-as compared to α_1-adrenoceptors [22,48]. Our studies showing that idazoxan's ability to enhance NE release was diminished as a function of diabetes, favor the concept that a direct relationship exists between α_2-adrenoceptor sensitivity and idazoxan action on NE release, at least as far as the lumbar segment of the spinal cord is concerned. The observation of diabetes-induced attenuation of idazoxan action on noradrenergic transmission is reminiscent of that seen with the analgesic and hyperalgesic action of clonidine and yohimbine, respectively [7,29]. Additional studies are in progress to establish a link between α_2-adrenoceptor function, NE release and GTP-binding proteins during diabetes. G-proteins serve as intermediaries in transmembrane signal transduction of α_2-adrenoceptor [10].

A wealth of evidence indicates that GCs are implicated in the regulation of noradrenergic systems within the CNS. In this context, previous reports have shown that chronic administration of a supraphysiological dose of GCs suppressed yohimbine-induced release of NE in the hypothalamus of conscious rats [47]. Similar data were obtained with idazoxan in lumbar segments of the spinal cord (present data). These findings suggest that hypercortisolemia negatively controls the expression of α_2-adrenoceptors. In total agreement with this premise, our current finding demonstrates a reduction in the binding sites and mRNA levels of α_2-adrenoceptor in non-diabetic hypercortisolemic animals. By contrast, an adrenal deficiency state appears to favor the expression of α_2-adrenoceptors [31,50]. In view of this information, it is possible to champion the notion that the attenuation in idazoxan's ability to enhance NE-release during diabetes is mediated by GC. Support for this premise is related to the previous experiment [3] and clinical data [11,51] that indicate that the diabetic state is associated with hypercortisolemia. Obviously, further studies aimed at counteracting the excessive action of GCs in diabetes are needed in order to support or refute the above hypothesis.

Neuropathy is a common feature of both clinical and experimental diabetes. This phenomenon is associated with a variety of aberrant sensations including spontaneous pain, allodynia and hyperalgesia [1]. Treatment such as topical capsaicin and oral mexiletine are occasionally effective in relieving pain but they exhibit a number of side effects, thus restricting their use clinically [12,19]. Recent data showed,

however, that certain symptoms of painful diabetic neuropathy such as mechanical and cold allodynia are under spinal noradrenergic control [69]. For example α_2-adrenergic agonists as well as tricyclic antidepressants (inhibitors of NE and serotonin reuptake) are effective in ameliorating neuropathic pain [23,70,71]. Overall, the present findings together with those reported previously suggest that diabetic and hypercortisolemic allodynic symptoms may stem at least in part from lumbospinal down-regulation of α_2-adrenoceptors. Obviously, further work is necessary to address the potential involvement of the various subtypes of α_2-adrenoceptors in the diabetic and hypercortisolemic models of neuronal sensitization. For example, would chronic intrathecal administration of an antisense oligodeoxynucleotide probe specific for a selective α_2-adrenoceptor subtype (e.g. α_{2A}-adrenoceptor) negate the anti-allodynic activity of clonidine or dexmedetomidine?

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References

[1] Benbow SJ, Chan AW, Bowsher D, MaCfarlane IA, Williams G. A prospective study of painful symptoms, small-fiber function and peripheral vascular disease in chronic painful diabetic neuropathy. Diabetes Med 1994;11:17–21.

[2] Biessels G, Cristino NA, Rutten G, Hamers FPT, Erkelens DW, Gispen WH. Neurophysiological changes in the central and peripheral nervous system of streptozotocin diabetic rats. Brain 1999; 122:757–68.

[3] Bitar MS. Glucocorticoid dynamics and impaired wound healing in diabetes mellitus. Am J Pathol 1998;152:547–54.

[4] Bitar MS, Bajic KT, Farook T, Thomas MI, Pilcher CWT. Spinal cord noradrenergic dynamics in diabetic and hypercortisolemic states. Brain Res 1999;830:1–9.

[5] Bitar MS, De Souza EB. Diabetes-related changes in brain beta adrenoceptors in rats as assessed by quantitative autoradiography: relationship to hypothalamic norepinephrine metabolism and pituitary gonadal hormone secretion. J Pharmacol Exp Ther 1990; 254:781–5.

[6] Bitar MS, Koulu M, Rapoport SI, Linnoila M. Diabetes-induced alterations in brain monoamine metabolism in rats. J Pharmacol Exp Ther 1986;236:432–7.

[7] Bitar MS, Pilcher CWT. Insulin dependent attenuation in of α_2 adrenoreceptor-mediated nociception in experimental diabetes. Pharmacol Biochem Behav 1997;56:15–20.

[8] Bowker RM, Westlund KN, Coulter JD. Origins of serotonergic projections to the spinal cord in rat: an immunocytochemical-retrograde transport study. Brain Res 1981;226:187–99.

[9] Braestrup C. Effects of phenoxybenzamine, aceperone and clonidine on the levels of 3-methoxy-4-hydroxyphenyl glycol in rat brain. J Pharm Pharmacol 1974;26:139–41.

[10] Brinbaumer L, Abramowitz J, Brown AM. Receptor-effector coupling by G. protein. Biochem Biophys Acta 1990;1031:163–224.

[11] Cameron OG, Tiongeo D, Thomas B, Greden J. Hypercortisolism in diabetes mellitus. Diabetes Care 1987;10:662–4.

[12] Capsaicin-study group. Effect of treatment with capsaicin on daily activities of patient with painful diabetic neuropathy. Diabetes Care 1992;15:159–65.

[13] Carsten RE, Whalen LR, Ishii DN. Impairment of spinal cord conduction velocity in diabetic rats. Diabetes 1989;38:730–6.

[14] Cedarbaumn JM, Aghajanian GK. Noradrenergic neurons of the locus coeruleus inhibition by norepinephrine and activation by the alpha antagonist piperoxane. Brain Res 1976;112:413–9.

[15] Chomczynski P, Sacchi N. Single-step method of RNA isolation by acid guanidinium thiocyanate phenol-chloroform extraction. Anal Biochem 1987;162:156–9.

[16] Courteix C, Eschalier A, Lavareane J. Streptozotocin-induced diabetic rats: behavioral evidence for a model of chronic pain. Pain 1993;53:81–8.

[17] Dalley JW, Stanford SC. Contrasting effects of the imidazoline α_2-adenoceptor agonists, medetomidine, clonidine and UK 14,304 on extraneuronal levels of noradrenaline in the rat frontal cortex evaluation using in vivo microdialysis and synaptosomal uptake studies. Br J Pharmacol 1995;114:1717–23.

[18] Danzebrink RM, Gebhart GF. Antinociceptive effects of intrathecal adrenoceptor agonists in a rat model of visceral nociception. J Pharmacol Exp Ther 1990;253:698–705.

[19] DeJgard A, Kastrup J, Petersen P. Mexiletine for treatment of chronic painful diabetic neuropathy. Lancet 1988;1:9–11.

[20] Dennis T, Heureux RL, Carter C, Scatton B. Presynaptic alpha-2 adrenoceptors play a major role in the effects of idazoxan on cortical noradrenaline release (as measured by in vivo dialysis). J Pharmacol Exp Ther 1987;241:642–9.

[21] Desbuquois D, Aurbach GD. Use of polyethylene glycol to separate free and antibody-bound peptide hormones in radioimmunoassays. J Clin Endocrinol Metab 1971;33:732–8.

[22] Dooxey JC, Roach AG, Smith CFC. Studies on RX 781094: a selective potent and specific antagonist of α2 adrenoreceptor. Br J Pharmacol 1983;78:484–505.

[23] Dubner R, Max M. Antidepressants and relief of neuropathic pain: evidence for noradrenergic mechanisms. In: Besson JM, Guilbaud G, editors. Noradrenergic agonists for the treatment of pain. Amsterdam: Excerpta Medica, 1992. pp. 211–8.

[24] Felten DL. Spinal cord alteration in streptozotocin-induced diabetes. Anat Rec 1979;193:741.

[25] Feteris WA. A serum glucose method without protein precipitation. Am J Med Technol 1965;31:17–21.

[26] Fields HL, Basbaum AL. Brain stem control of spinal pain transmission. Neurons Ann Rev Physiol 1978;40:217–48.

[27] Gjerd H, Morland J, Olsen H. The antiglucocorticcoid RU 486 inhibit the ethanol-induced increase of tryptophan oxygenase. J Steroid Biochem 1985;23:1091–2.

[28] Greene DA, Sima AAF, Feldman EL, Stevens MJ. Diabetic neuropathy. In: Rifkin H, Porte D, Sherwin R, editors. Ellenberg and Rifkin diabetes mellitus. Stamford, CT: Appleton and Lange, 1997. pp. 1009–76.

[29] Guo T, Maze B, Maze M. Attenuation of central α_2 adrenergic action in diabetic rats. Pharmacol Biochem Behav 1991;39:383–7.

[30] Hammond DL, Tyce GM, Yaksh TL. Efflux of 5-hydroxytryptamine and noradrenaline into the spinal cord superfusates during stimulation of the rat medulla. J Physiol 1985;359(London):151–62.

[31] Jhanwar-Uniyal M, Leibowitz SF. Impact of circulating corticosterone on α_1 and α_2 adrenergic receptors in discrete brain areas. Brain Res 1986;368:404–8.

[32] Kamisaki Y, Ishikaawa T, Takao Y, Omadani H, Kuno N, Itoh T. Binding of [^{3}H] *p*-aminoclonidine to brain sites, α_2-adrenoceptor and imidazoline binding sites: distribution of imidazoline binding sites in rat brain. Brain Res 1990;514:15–21.

[33] Knox WF, Auerback VH. The hormonal control of tryptophan peroxidase in the rat. J Biol Chem 1955;214:307–13.
[34] Lanier SM, Downing S, Duzic E, Homcy CJ. Isolation of rat genomic clones encoding subtypes of the α_2-adrenergic receptors. J Biol Chem 1991;266:10470–8.
[35] Lawhead RG, Blaxall HS, Bylund DB. α_{2A} is the predominant α_2-adrenoceptor subtype in human spinal cord. Anesthesiology 1992;77:983–91.
[36] Lode HN, Bruchett G, Seitz G, Gebhardt S, Gekeler V, Niethamer D, Beck J. Reverse-trancriptase–polymerase chain reaction (RT–PCR) analysis of monoamine transporters in neuroplastoma cell lines: correlations to meta-idobenzyl guanidine up take and tyrosine hydroxylase gene expression. Eur J Cancer 1995;31:586–90.
[37] Lookingland KJ, Ireland LM, Gunnet JW, Manzawares J, Tian Y, Moore KE. 3-Methoxy-4-hydroxypheneyl glycol concentrations in discrete hypothalamic nuclei reflect the activity of noradrenergic neurons. Brain Res 1991;559:82–8.
[38] Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the folin phenol reagent. J Biol Chem 1951;139:265–75.
[39] Maze M, Poreeq L, Rabin BC. Anesthetic and analgesic actions of α_2-adrenoceptor agonist. Pharmacol Commun 1995;6(Suppl):175–82.
[40] Maze M, Tranquilli W. Alpha-2 adrenoceptor agonists defining the role in clinical anesthesia. Anesthesiology 1991;74:581–605.
[41] Meert TF, Dekock M. Potentiation of the analgesic properties of fentanyl-like opioids with α_2-adrenoceptor agonists in rats. Anaesthesiology 1994;81:677–88.
[42] Mendez R, Eisenach JC, Kashtan K. Epidural clonidine analgesia after caesarian section. Anaesthesiology 1990;73:848–52.
[43] Millan MJ, Bervoets K, Rivet JM, Widdowson P, Renouard A, Le-Maroulie Girardon S, Gobert A. Multiple α_2-adrenoceptors in the control of nociception motor behaviour and hippocampal synthesis of noradrenaline. J Pharmacol Exp Ther 1994;270:958–72.
[44] Nicholas AP, Hokfelt T, Pieribone VA. The distribution and significance of CNS adrenoceptor examined with in situ hybridization. TIPS 1996;17:245–55.
[45] Nygen LG, Olson L. A new major projection from locus coeruleus: the main source of noradrenergic treatments in the vertical and dorsal columns of the spinal cord. Brain Res 1977;132:85–93.
[46] Ohnishi A, Harada M, Tateishi J, Ogata J, Kawanami S. Segmental demyelination and remyelination in lumber spinal roots of patient dying with diabetes mellitus. Ann Neurol 1983;13:541–8.
[47] Pacak K, Armando I, Komoly S, Fukuhara K, Weise VK, Holmes C, Kopin IJ, Goldstein DS. Hypercortisolemia inhibits yohimbine-induced release of norepinephrine in the posterolateral hypothalamus of conscious rats. Endocrinology 1992;131:1369–76.
[48] Preziosi P, Martire M, Navarra P, Pistritto G, Vaca M. Prolactin-lowering ability of (±) idazoxan may be linked to a central noradrenergic/serotonergic interplay. J Pharmacol Exp Ther 1989;249:256–63.
[49] Puke MJ, Luo L, Xu XJ. The spinal analgesic role of α_2-adrenoceptor subtypes in rats after peripheral nerve section. Eur J Pharmacol 1994;260:227–32.
[50] Robinson JP, Kendall DA. The influence of adrenalectomy on alpha-adrenoceptor response in rat cerebral cortex slices. Mol Brain Res 1990;7:69–74.
[51] Roy M, Collier B, Roy A. Hypothalamic–pituitary adrenal axis dysregulation among diabetic out patients. Psychol Res 1990;31:31–7.
[52] Ruffolo RR, Nichols AJ, Stadel JM, Hieble JP. Structure and function of alpha-adrenoceptors. Pharmacol Rev 1991;43:475–505.
[53] Savola MKT, Savola J-M. $\alpha_{2A/D}$-adrenoceptor subtype predominates also in the neonatal rat spinal cord. Dev Brain Res 1996;94:106–8.
[54] Sevnnson TH, Bunney BS, Aghajanian GK. Inhibition of both noradrenergic and serotonergic neurons in brain by the alpha adrenergic agonist clonidine. Brain Res 1975;291:291–306.
[55] Starke K. Presynaptic α-autoreceptors. Rev Physiol Biochem Pharmacol 1987;107:73.
[56] Stone EA. Effect of stress on sulfated glycol metabolite of brain norepinephrine. Life Sci 1975;16:1725–30.
[57] Thomas DN, Holman RM. Effects of acute and chronic electroconvulsive shock on noradrenaline release in the rat hippocampus and frontal cortex. Br J Pharmacol 1991;106:430–4.
[58] Thomas PK, Tomlinson DR. Diabetic and hypoglycemic neuropathy. In: Dyck PJ, Thomas PK, Gruffin JW, Low PA, Poduslo JF, editors. Peripheral neuropathy. Philadelphia: Saunders, 1993. pp. 1219–50.
[59] Uhlen S, Wikberg JE. Rat spinal cord alpha-2 adrenoceptors are of the alpha 2A-subtype. Comparison with alpha 2A- and alpha 2B-adrenoceptors in rat spleen, cerebral cortex and kidney using 3H-RX 821002 ligand binding. Pharmacol Toxicol 1991;69:341–50.
[60] Umeda E, Satoh T, Potter PE, Tarkovacs G, Vizi S. α_{2A} subtype of presynaptic, α_2-adrenoceptor modulates the release of [^{3}H] noradrenaline from rat spinal cord. Brain Res Bul 1997;42:129–32.
[61] Unnerstall JR, Kopajtic TA, Kuhar MJ. Distribution of α-agonist binding sites in the rat and human CNS: analysis of some functional autonomic correlates of the pharmacological effects of clonidine and related adrenergic agents. Brain Res Rev 1984;19:69–101.
[62] Van Veldhuizen MJA, Feenstra MGP, Heinsbroek RPW, Boer GJ. In vivo microdialysis of noradrenaline overflow: effects of α-adrenoceptor agonists and antagonists measured by accumulative concentration–response curves. Br J Pharmacol 1993;109:655–60.
[63] Viniki A, Mitchell B. Clinical aspects of diabetic neuropathies. Diabetes/Metab Rev 1988;4:223–53.
[64] Vizi ES. Presynaptic modulation of neurochemical transmission. Prog Neurobiol 1979;12:181–290.
[65] Ward JD. Diabetic neuropathy. Br Med Bull 1989;45:111–26.
[66] Warsh JJ, Li PP, Godse DD, Cheung S. Brain noradrenergic neuronal activity affects 3,4 dihydroxyphenylethylglycol levels. Life Sci 1981;29:103–7.
[67] West W, Yeomans DC, Proudfit HK. The function of noradrenergic neurons in mediating antinociception induced by electrical stimulation of the locus coeruleus in the different sources of Sprague Dawley rats. Brain Res 1993;626:127–35.
[68] Westlund KN, Bowker RM, Ziegler MG, Coulter JD. Noradrenergic projections to the spinal cord of the rat. Brain Res 1983;263:15–31.
[69] Xu M, Kontinen VK, Kalso E. Endogenous noradrenergic tone controls symptoms of allodynia in the spinal nerve ligation model of neuropathic pain. Eur J Pharmacol 1999;366:41–5.
[70] Yaksh TL, Pogrel JW, Lee YW, Chaplan SR. Reversal of nerve ligation-induced allodynia by spinal α_2-adrenoceptor agonists. J Pharmacol Exp Ther 1995;272:207–14.
[71] Zeigler D, Lynch SA, Muir J, Benjamin J, Max MB. Transdermal clonidine vs. placebo in painful diabetic neuropathy. Pain 1992;48:403–8.
[72] Zimmerman M. Ethical guidelines for investigations of experimental pain. Pain 1983;16:109–10.