Pharmacology Biochemistry and Behavior, Vol. 56, No. 1, pp. 15–20, 1997

0091-3057/97 $17.00 + .00

PII S0091-3057(96)00129-3

Insulin-Dependent Attenuation in α_2-Adrenoreceptor–Mediated Nociception in Experimental Diabetes

MILAD S. BITAR[1] AND CHARLES WILLIAM THOMAS PILCHER

Department of Pharmacology and Toxicology, School of Medicine, Kuwait University, 13110 Safat, Kuwait

Received 25 July 1995; Revised 26 January 1996; Accepted 9 February 1996

BITAR, M. S. AND C. W. T. PILCHER. *Insulin-dependent attenuation in α_2-adrenoreceptor–mediated nociception in experimental diabetes.* PHARMACOL BIOCHEM BEHAV **56**(1) 15–20, 1997.—Diabetes mellitus is associated with abnormalities in central noradrenergic dynamics, a system that appears to be involved in the regulation of nociception in both humans and experimental animals. To this end, we investigated the responsiveness of nociceptive threshold to the actions of clonidine (an α_2-adrenoreceptor agonist) and yohimbine (an α_2-adrenoreceptor antagonist) during diabetes. The induction of diabetes was achieved by the administration of streptozotocin (STZ) (55 mg/kg, intravenously). Nociceptive threshold, as indicated by the tail-flick latency of the tail immersion test, was progressively elevated as a function of the duration of diabetes. Systemic administration of clonidine and yohimbine respectively produced dose-dependent analgesic and hyperalgesic effects in control animals. Both of these phenomena were impaired in chronically diabetic animals. In contrast, insulin-treated diabetics displayed supersensitivity to clonidine's antinociceptive effect, especially at low doses. Acute hyperglycemia did not interfere with the α_2-agonist–mediated elevation in nociceptive threshold. Attenuation in clonidine antinociceptive effect was also observed following its intrathecal administration to diabetic animals. Overall, these data suggest that the impaired responsiveness of diabetic rats might be due to a central α_2-adrenoreceptor desensitization and/or biochemical defect in the postreceptor events. **Copyright © 1997 Elsevier Science Inc.**

Experimental diabetes Insulin Clonidine Yohimbine Nociception

α_2-ADRENERGIC agonists, including clonidine, medetomidine, and ST91 have been shown to elevate nociceptive thresholds and potentiate the analgesic effect of opioids both in humans and experimental animals (8,22–25,27,28). In addition, these compounds also reduced the general anaesthetic requirement during surgery and prolonged tetracaine- and bupivacaine-induced spinal anaesthesia [(7,18; for review see (21)]. Although most of these pharmacologic actions appear to be mediated by the activation of central α_2-adrenergic receptors (12,21,35), the potential interaction of α_1-adrenoreceptors in the processing of nociceptive information cannot be ruled out at this time.

Central noradrenergic processes are altered as a function of diabetes. In this context, the activity of tyrosine hydroxylase, the rate-limiting enzyme in catecholamine biosynthesis, and norepinephrine (NE) turnover rate are decreased in specific brain areas of diabetic animals (2,4). In contrast, an increase in the densities of α_1- and β_2-adrenoreceptor binding sites were observed in these animals (3,4). In view of diabetes-related abnormalities in noradrenergic dynamics and the well-established role of this system in controlling nociceptive responsiveness, we investigated the effects of the α_1-adrenoreceptor agonist clonidine and the antagonist yohimbine on pain thresholds in streptozotocin (STZ)-treated rats, an animal model for type I diabetes mellitus.

METHOD

Animals

Adult female Sprague–Dawley rats (Kuwait University breeding colony) weighing 200–220 g were individually housed on a 12 L:12 D cycle (lights on from 0600 to 1800 h). The ambient temperature was kept at 21°C and the rats had free access to standard laboratory food and tapwater.

[1]Requests for reprints should be addressed to M. S. Bitar, Department of Pharmacology and Toxicology, Faculty of Medicine, Kuwait University, Post Box 24923, 13110 Safat, Kuwait.

Treatment

Diabetes was induced by an intravenous (IV) injection of STZ (55 mg/kg body wt.) diluted in 0.05 M sodium citrate, 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, and the other receiving subcutaneous (SC) injection of crystalline zinc insulin and lente insulin (1:3 ratio), 5–8 U daily, starting 3 days after STZ injection and continuing throughout the experimental period. The dose of insulin was determined on the basis of the daily urine and weekly blood tests for glucose.

Surgery

Rats were anaesthesized with pentobarbital [50 mg/kg, intraperitoneally (IP)], and the intrathecal catheters were implanted as described previously (37). In brief, the animals were positioned in a stereotactic apparatus and a fine polyethylene-10 catheter was inserted into the subarachnoid space via the atlanto-occipital membrane. The catheter extended to the rostral end of the lumbar enlargement at the L_1–L_2 level. Immediately and 24 h after placement, catheters were flushed with 10 μl of sterile saline. Animals were carefully checked for spinal cord damage by evaluation of motor behavior and examination of limb, body, and tail posture. Only rats displaying no signs of spinal cord damage were included in the study. We allowed 4–6 days for recovery before testing. The drug was dissolved in sterile saline and infused in a volume of 10 μl followed by a flush of 10 μl saline.

Nociceptive Testing

All nociceptive latencies were determined using the tail withdrawal reaction (TWR) assay. A detail regarding this procedure was outlined previously (15). In brief, the test was conducted by immersing the rat tail to a depth of 5 cm in water maintained at 48°C. The nociceptive end point was characterized by a violent jerk of the tail (tail-flick/withdrawal). Baseline values were 5–10 s, and a 25-s cutoff was imposed. For each rat, the nociceptive threshold was determined before and 30 min after clonidine, yohimbine, or saline administration. In the antagonism experiment, however, yohimbine was administered IP 15 min before clonidine treatment and the tail immersion latencies were measured 45 min afterward (i.e., 30 min following clonidine injection). A time-response function was established for the analgesic and hyperalgesic actions of clonidine and yohimbine, respectively. The data revealed that both drugs attain their peak effects at 30 min after SC and IP administration.

Drugs

Clonidine was dissolved in saline or water, whereas 2.5% DMSO in water or saline was used to dissolve yohimbine. A volume of 1 ml/kg body wt. of drug solution was used for each injection. All drugs were freshly dissolved before each experiment and injected SC or IP.

Glucose

Plasma glucose concentrations were measured using the *o*-toluidine method (11).

Statistical Procedures

Postdrug thresholds were calculated as a percentage of change from the baseline. Statistical calculations were done with a one-way analysis of variance followed by a two-tailed *t*-test. Results are expressed as means ± SEM and were regarded as significant when $p < 0.05$. For analysis, TWR latencies were converted to percent maximum possible effect (MPE), according to the formula:

$$\%\text{MPE} = \frac{\text{TWR latency with drug} - \text{Baseline latency}}{\text{Cutoff time (25 s)} - \text{Baseline latency.}}$$

RESULTS

Changes in body weights and plasma glucose concentrations at various time intervals following diabetes induction are presented in Table 1. The body weights of the 2-, 4-, and 8-week diabetic animals were decreased by 13%, 19%, and 22%, respectively, from corresponding controls. A further decrease (28%) in body weight was observed in the 12-week diabetic rats. In contrast, plasma glucose concentrations in diabetic animals were significantly higher than control at each time interval of diabetes duration. The decrease in body weights and the increase in plasma glucose levels were restored toward normal values following the daily injection of insulin.

Data concerning the effect of duration of diabetes on nociceptive threshold in the tail immersion test are presented in Fig. 1. The nociceptive threshold, as indicated by the tail-flick/withdrawal latency of the tail immersion test, showed progressive elevation as a function of the duration of diabetes. For example, the nociceptive threshold of the 2-week diabetic rats was elevated by 22% from its corresponding control value. The magnitude of this elevation in nociceptive threshold was even greater in animals with diabetes of longer duration. In contrast, the nociceptive threshold in insulin-treated diabetes was significantly lower than corresponding control or diabetic values.

Data concerning nociceptive threshold in response to various doses of clonidine are presented in Fig. 2. In control animals, clonidine produced a dose-dependent elevation in nociceptive threshold. This antinociceptive action of clonidine was impaired in diabetic animals. Indeed, only the 1-mg/kg dose of clonidine was capable of elevating nociceptive threshold by 35% of MPE. In contrast, the insulin-treated diabetic group exhibited supersensitivity to the analgesic action of clonidine, especially at low doses. For example, clonidine administered at a dose of 0.1 mg to insulin-treated diabetics elevated nociceptive threshold by 15% vs. 1.3% (control).

Figure 3 demonstrates the effect of acute hyperglycemia on the antinociceptive action of clonidine. Acute hyperglycemia (e.g., 447 ± 15 mg/100 ml of plasma) induced by the IP administration of *D*-glucose (20 mmol in 2.5 ml saline) did not interfere with clonidine's ability to elevate nociceptive threshold. Indeed, the degree of elevation in nociceptive threshold after the administration of 400 μg/kg clonidine was similar in control and acutely hyperglycemic animals.

To determine whether the impairment of clonidine's antinociceptive action in diabetes was due to a pharmacodynamic or pharmacokinetic mechanism, we measured nociceptive threshold in control and diabetic animals following intrathecal administration of this drug. The data in Fig. 4 clearly indicate that in the 1-week diabetic rats, the antinociceptive effect of clonidine was similar to that of control. However, in the 12-week diabetic rats, the magnitude of clonidine-induced elevation in nociceptive threshold was only 11% of predrug values.

Table 2 shows the effects of various doses of yohimbine on nociceptive threshold in control and 90-day diabetic rats.

TABLE 1

BODY WEIGHT AND PLASMA GLUCOSE AS A FUNCTION OF THE DURATION OF DIABETES

Duration of Diabetes	Body Weight			Plasma Glucose (mg/100 ml)		
	Control	Diabetic + Insulin	Diabetic	Control	Diabetic + Insulin	Diabetic
1 week	225 ± 7.0	217 ± 8.3	222 ± 7.3	122 ± 12	336 ± 28*	187 ± 13
2 weeks	238 ± 6.0	207 ± 7.4	230 ± 5.0	125 ± 7	396 ± 31*	202 ± 9
4 weeks	246 ± 5.2	206 ± 9.2*	237 ± 5.8	115 ± 9	463 ± 41*	177 ± 15
8 weeks	258 ± 5.7	200 ± 8.7*	260 ± 6.3	128 ± 11	448 ± 33*	158 ± 23
12 weeks	270 ± 6.5	194 ± 12.6*	277 ± 5.9	119 ± 8	510 ± 38*	215 ± 27

STZ was injected IV, 55 mg/kg in 0.05 M citrate buffer, pH 4.5. Values are expressed as means ± SEM for at least 10 animals.

*Significantly different from control or insulin-treated diabetics.

Yohimbine produced a dose-dependent reduction in nociceptive threshold when administered SC to control animals. This hyperalgesic effect of yohimbine was not seen in the 90-day diabetic rats. Indeed, even at a dose of 5 mg/kg, yohimbine decreased nociceptive threshold in diabetic rats by only 12%. This was not significantly different compared with predrug values.

Table 3 shows the results of an experiment designed to investigate whether the antinociceptive effect of clonidine is mediated by α2-adrenoreceptors. Yohimbine administered at 1.25 mg/kg, a dose which does not alter nociceptive threshold, completely prevented clonidine-induced antinociceptive effects.

DISCUSSION

This study represents the first part of an investigation aimed at examining the behavioural, therapeutic, and pharmacodynamic aspects of α2-adrenoreceptor function during diabetes. Tail-flick latency in the tail immersion assay was used as an indicator for the changes in spinal nociception in response to diabetes and the α2-adrenoreceptor ligands, including clonidine and yohimbine. STZ-treated rats were used because this animal model exhibits neurochemical and hormonal abnormalities in many respects resembling that of human diabetes (2,4,20,31).

The central finding of this report is that CNS α2-adrenoreceptor–mediated alteration in nociceptive threshold is progressively attenuated as a function of the duration of diabetes. In this vein, clonidine and yohimbine respectively produced dose-dependent analgesic and hyperalgesic effects when administered to normal animals. However, diminution in the ability of these compounds to alter nociceptive threshold was evident in chronically diabetic rats. Diabetic animals also exhibited an elevation in nociceptive threshold against thermal but not mechanical stimuli (unpublished observation). In this respect, our data regarding the elevation in nociceptive threshold in diabetic animals, together with the dose-dependent analgesic and hyperalgesic effects of clonidine and yohimbine, are

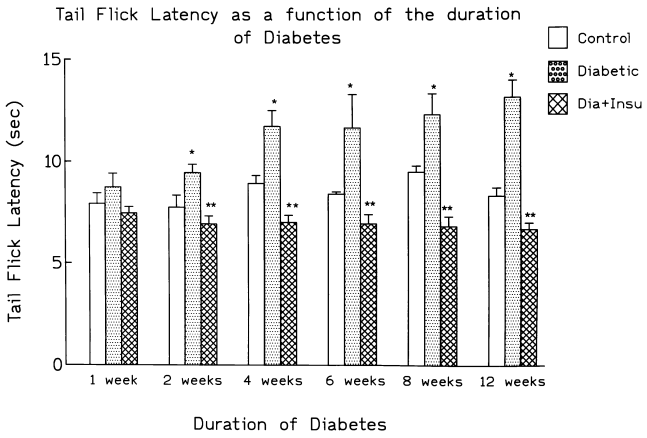

FIG. 1. Tail-flick latency as a function of the duration of diabetes. Rats were treated with STZ 55 mg/kg or citrate buffer (control). Three days after diabetes induction, replacement therapy with insulin (lente insulin/crystalline zinc insulin, 3:1) was instituted at a dose of 5–7 U/day for the duration of the experiment (12 weeks). Tail withdrawal latency in the tail immersion test was recorded with a stimulus temperature of 48°C. Values are expressed as means ± SEM; $n \geq 8$. * Significantly different from corresponding vehicle-treated values at $p < 0.05$. ** Significantly different from control and diabetic values at $p < 0.05$.

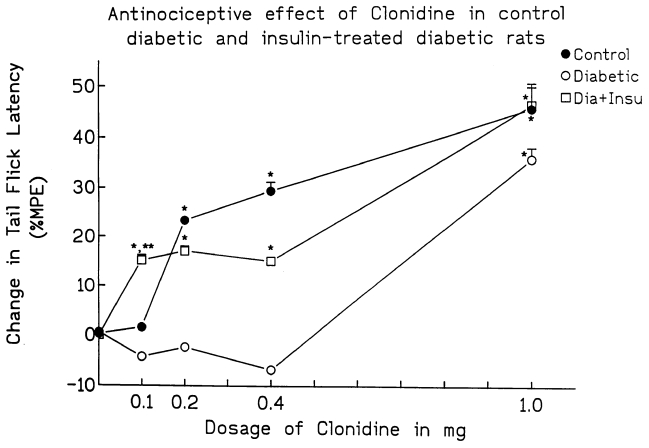

FIG. 2. Antinociceptive effect of clonidine in control, diabetic, and Insulin-treated diabetic rats. Rats were treated with STZ, 55 mg/kg, IV, or citrate buffer (control). Three days after diabetes induction, insulin treatment (lente insulin/crystalline zinc insulin, 3:1) was instituted at a dose of 6 U/day for the duration of the experiment (12 weeks). Tail withdrawal latency in the tail immersion test was recorded 30 min after the vehicle (0) or various doses of clonidine (0.1 mg to 1 mg/kg). The stimulus temperature was 48°C. Values expressed as means ± SEM; $n \geq 8$/data point. *Significantly different from vehicle-treated group at $p < 0.05$. **Significantly different with regard to clonidine sensitivity from control and diabetic animals at $p < 0.05$.

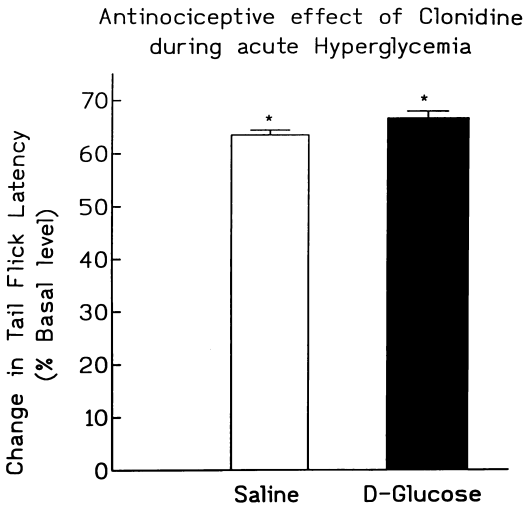

FIG. 3. Antinociceptive effects of clonidine during acute hyperglycemia. Acute hyperglycemia was induced by the IP administration of D-glucose at a dose of 20 mmol in 2.5 ml of saline. Control animals received saline alone by the same route. Tail-flick latency in the tail immersion test was recorded 60 min (preclonidine) and 90 min (postclonidne, 0.4 mg/kg) after D-glucose administration. Data are expressed as a percentage of means of preclonidine values. *Significantly different from preclonidine values at $p < 0.05$.

consistent with previously published observations (1,27,29,36). However, the hyporesponsiveness of α2-adrenoreceptors to the actions of clonidine and yohimbine in chronically diabetic animals, which may more closely reflect the situation in humans, is novel.

These biochemical and behavioural abnormalities are not likely to be due to a direct toxic effect of STZ, because the dose employed acts selectively on β-cells (16,17). The most compelling evidence, however, was that insulin replacement therapy of diabetic animals reversed the elevation in nociceptive threshold and ameliorated the impairment of clonidine's antinociceptive effect.

The ability of clonidine to elevate nociceptive threshold appears to be impaired as a function of the duration of diabetes (Fig. 2). Likewise, blunted diuretic and natriuretic responses to centrally administered clonidine were also observed in this disease state (39). Diabetes-induced resistance to clonidine's actions is not unique to this drug, as a similar decrease in the analgesic action of opiates has been reported in experimental and human diabetes (26,32,33). In addition, other compounds including yohimbine, salbutamol, and dexmedetomidine, respectively mediating hyperalgesic, anorexigenic, and hypnotic actions, were also attenuated during diabetes (Table 3) (14,25). Taken together, these data support the premise that the CNS adrenergic mechanism is hypoactive during chronic diabetes.

Drug biotransformation and pharmacokinetics are altered as a function of diabetes (5,6,18). Accordingly, an attempt was made to determine whether a pharmacokinetic and/or pharmacodynamic mechanism is involved in diabetes-related impairment of clonidine's antinociceptive effects. Our data showed significant attenuation in the ability of clonidine to elevate nociceptive threshold after its intrathecal administration to chronically diabetic rats, thus supporting the notion that the hyporesponsiveness to the analgesic action of clonidine in diabetes is centrally mediated. However, the possibility of diabetes-related alterations in clonidine pharmacokinetics cannot be excluded at this time.

Several lines of evidence have indicated that central monoaminergic systems exert a tonic inhibitory effect on nociception (29,30,34,36). In this context, the administration of monoamine antagonists (yohimbine and idazoxan) or selective lesioning of noradrenergic pathways reduces the response latencies in nociceptive tests such as the hot-plate and tail-flick tests (Table 3) (29,34). In contrast, the administration of α_2-adrenoreceptor agonists (e.g., clonidine, dexmedtomidine, ST91) produce antinociception (12,21,35). Given this evidence, it is reasonable to speculate that diabetes-induced resistance to the analgesic and hyperalgesic effects of clonidine and yohimbine, respectively, reflects abnormalities in adrenergic mechanism within the CNS. Partial support of this premise is related to our previous studies showing that central noradrenergic mechanism is hypoactive in STZ diabetic rats (2,4). Stud-

TABLE 2

HYPERALGESIC EFFECTS OF YOHIMBINE IN CONTROL AND 90-DAY DIABETIC RATS

	Tail-Flick Latency as % of Basal	
Dose (mg/kg)	Control	90-day Diabetic
0	100 ± 6	100 ± 8
1.25	97 ± 4	92 ± 4
2.50	81 ± 6*	87 ± 6
5.00	69 ± 3*	88 ± 7

Rats were treated with STZ, 55 mg/kg, IV, or citrate buffer (control). Tail withdrawal latency in the tail immersion test was recorded 30 min after the vehicle (0) or various doses of yohimbine. The stimulus temperature was 48°C. Values expressed as means ± SEM; n ⩾ 8.

*Significantly different from vehicle-treated group at $p < 0.05$.

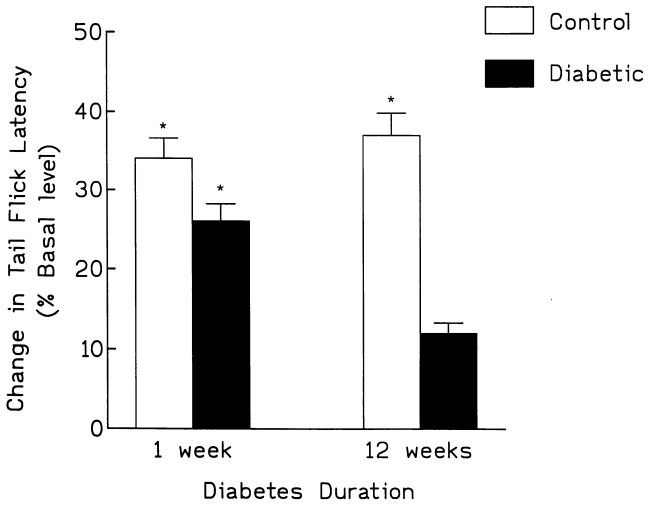

FIG. 4. Antinociceptive effect of intrathecal administration of clonidine after STZ treatment. Rats were treated with STZ, 55 mg/kg, IV, or citrate buffer (control). Nociceptive threshold was determined 1 and 12 weeks after diabetes induction. Clonidine was administered intrathecally in a volume of 10 ml and at a dose of 10 mg/animal. Data are expressed as a percentage of means of predrug values ± SEM. *Significantly different from predrug value.

TABLE 3

EFFECTS OF YOHIMBINE ON CLONDINE-INDUCED ELEVATION OF NOCICEPTIVE THRESHOLD OF CONTROL AND 90-DAY DIABETIC RATS

	Tail-Flick Latency (s)			
	Control		Diabetic	
Treatment	Saline	Clonidine	Saline	Clonidine
Vehicle	7.46 ± 0.43	9.78 ± 0.68*	8.93 ± 0.53	9.32 ± 0.71
Yohimbine	7.43 ± 0.41	7.62 ± 0.65	9.43 ± 0.31	9.10 ± 0.73

Yohimbine was administered IP at a dose of 1.25 mg/kg, 15 min prior to treatment with 0.2 mg/kg of clonidine. Tail-flick latency in the tail immersion test was recorded 30 min thereafter; values represent the means ± SEM for at least 10 animals.

* Significantly different from saline-injected control animals.

ies are being conducted in our laboratory with the aim of elucidating the effect of diabetes on the status of α_2-adrenoreceptors (e.g., number, affinity) and various electrical and chemical consequences which result from the activation of these receptors (e.g., GTP-binding proteins, suppression of voltage-gated Ca^{2+} currents, activation of receptor-operated K^+ currents).

In conclusion, this study reports a hyporesponsiveness to clonidine's antinociceptive effect in chronically diabetic rats, a phenomenon which may be related to a defect in central α_2-adrenoreceptor and its second-messenger system. In conjunction with previous reports (2–4), our data support the notion of extensive changes in central noradrenergic mechanism(s) in diabetes. From a practical point of view, these results suggest that diabetic subjects who suffer from pain associated with neuropathy could be resistant to α_2-agonists, at least in poor metabolic control states. A case in point is the finding that treatment with clonidine, which has been reported to relieve postoperative pain (23), cancer pain (10), pain due to arachonoiditis (13), and sympathetically maintained pain (9) did not exert a significant antinociceptive effect in patients suffering from painful diabetic neuropathy (38), although these authors suggest the possibility that there is a clonidine-sensitive subset of patients.

ACKNOWLEDGEMENTS

The authors thank M. Thomas for expert technical assistance, and the research divisions of UpJohn Company for their generous gift of STZ.

REFERENCES

1. Akunne, H.; Soliman, K. F. The role of opioid receptors in diabetes and hyperglycemia-induced changes in pain threshold in the rat. Psychopharmacology (Berlin) 93:167–172; 1987.
2. Bitar, M. S.; DeSouza, E. B. Diabetes-related changes in brain beta adrenoreceptors in rats as assessed by quantitative autoradiography: Relationship to hypothalamic norepinephrine metabolism and pituitary gonadal hormone secretion. J . Pharmacol. Exp. Ther. 254:781–785; 1990.
3. Bitar, M. S.; Keis, A. F.; Owusu, S. E.; Mulven, R.; DeSouza, E. B. Impairment of albuterol-induced suppression of food intake in diabetes mellitus. Pharmacol. Biochem. Behav. 41:483–487; 1992.
4. Bitar, M. S.; Koulu, M.; Rapoport, S. I.; Linnoila, M. Diabetes-induced alterations in brain monoamine metabolism in rats. J. Pharmacol. Exp. Ther. 236:432–437; 1986.
5. Bitar, M. S.; Weiner, M. Diabetes-induced metabolic alterations in heme and various heme-containing enzymes in female rats. Diabetes 33:37–44; 1984.
6. Bitar, M. S.; Weiner, M. Heme and hemoproteins in streptozotocin diabetic female rats. Biochem. Pharmacol. 32:1921–1928; 1983.
7. Bonnet, F.; Brun-Buisson, V.; Francois, Y.; Catoire, P.; Saada, M. Effects of oral and subarachnoid clonidine on spinal anaesthesia with bupivacaine. Reg. Anaesth. 15:211–214; 1990.
8. Danzebrink, R. M.; Gebhart, G. F. Antinociceptive effects of intrathecal adrenoreceptor agonists in a rat model of visceral nociception. J. Pharmacol. Exp. Ther. 253:698–705; 1990.
9. Davis, K. D.; Treede, K. D.; Raja, S. N.; Meyer, R. A.; Campbell, J. N. Topical application of clonidine relieves hyperalgesia in patients with sympathetically maintained pain. Pain 47:309–318; 1991.
10. Eisenach, J. C.; Rauck, R. L.; Buzzenell, C.; Lysak, S. Z. Epidural clonidine analgesia for intractable cancer pain: Phase I. Anaesthesiology 71:647–652; 1989.
11. Feteris, W. A. A serum glucose method without protein precipitation. Am. J. Med. Technol. 31:17–21; 1965.
12. Franz, D. N.; Hare, B. D.; McCloskey, K. L. Spinal sympathetic neurons: Possible site of opiates-withdrawal suppression by clonidine. Science 215:1643–1643; 1982.
13. Glynn, C.; Dawson, D.; Sanders, R. A double-blind comparison between epidural morphine and epidural clonidine in patients with chronic noncancer pain. Pain 34:123–128; 1988.
14. Guo, T.; Maze, B.; Maze, M. Attenuation of central α_2 adrenergic action in diabetic rats. Pharmacol. Biochem. Behav. 39:383–387; 1991.
15. Janssen, P. A. J.; Niemegeers, C.; Dony, J. The inhibitory effects of fentanyl and other morphine-like analgesics on the warm water induced tail withdrawal reflex in rats. Arzneimittelforschung 13:502–507; 1963.
16. Junod, A.; Lambert, A. E.; Orci, L.; Pictet, R.; Gomet, A. S.; Renold, A. E. Studies of the diabetogenic action of streptozotocin. Proc. Soc. Exp. Biol. Med. 126:201–205; 1969.
17. Junod, A.; Lambert, A. E.; Stauffacher, W; Renold, A. E. Diabetogenic action of streptozotocin: Relationship of dose to metabolic response. J. Clin. Invest. 48:2129–2139; 1969.
18. Kato, R. Drug metabolism under pathologic and abnormal physiological states in animals and man. Xenobiotics 7:25–92; 1977.
19. Koukinen, S.; Pyykko, K. The potentiation of halothane anaesthesia by clonidine. Acta Anaesthesiol. Scand. 23:107–111; 1979.
20. Lackovic, Z.; Salkovic, M.; Kuci, Z.; Relja, M. Effect of long-

lasting diabetes mellitus on rat and human brain monoamines. J. Neurochem. 54:143–147; 1990.
21. Maze, M.; Poreeq, L.; Rabin, B. C. Anaesthetic and analgesic actions of α_2-adrenoreceptor agonists. Pharmacol. Commun. 6(Suppl):175–182; 1995.
22. Meert, T. F.; Dekock, M. Potentiation of the analgesic properties of fentanyl-like opioids with α_2-adrenoreceptor agonists in rats. Anaesthesiology 81:677–688; 1994.
23. Mendez, R.; Eisenach, J. C.; Kashtan, K. Epidural clonidine analgesia after caesarian section. Anaesthesiology 73:848–852; 1990.
24. Millan, M. J.; Bervoets, K.; Rivet, J. M.; Widdowson, P.; Renouard, A.; Le Marouille; Girardon, S.; Gobert, A. Multiple α_2 adrenergic receptors in the control of nociception, motor behaviour and hippocampal synthesis of noradrenaline. J. Pharmacol. Exp. Ther. 270:958–972; 1994.
25. Monasky, M. S.; Zinsmeister, A. R.; Stevens, C. W.; Yaksh, T. L. Interaction of intrathecal morphine and ST91 on antinociception in the rat: Dose-response analysis, antagonism and clearance. J. Pharmacol. Exp. Ther. 254:383–392; 1990.
26. Morley, G. K.; Mooradian, A. D.; Levine, A. S.; Morely, J. E. Mechanism of pain in diabetic peripheral neuropathy: The effect of glucose on pain perception in humans. Am. J. Med. 77:79–83; 1984.
27. Nakamura, M.; Ferreira, S. H. Peripheral analgesic action of clonidine: Mediation by release of endogenous enkephalin-like substances. Eur. J. Pharmacol. 146:223–228; 1988.
28. Ossipov, M. H.; Harris, S.; Lloyd, P.; Messineo, E. An isobolographic analysis of the antinociceptive effect of systemically and intrathecally administered combinations of clonidine and opiates. J. Pharmacol. Exp. Ther. 255:1107–1116; 1990.
29. Paalzow, G. H. M.; Paalzow, L. K. Yohimbine both increases and decreases nociceptive threshold in rats: Evaluation of the dose–response relationship. Naunyn-Schmied. Pharmacol. 332:193–197; 1983.
30. Proudfit, H. K. Pharmacological evidence for the modulation of nociception by noradrenergic neurons. Prog. Brain Res. 77:357–370; 1988.
31. Roy, M.; Collier, B.; Roy, A. Hypothalamic-pituitary adrenal axis dysregulation among diabetic and out-patients. Psych. Res. 31:31–37; 1990.
32. Simon, G. S.; Borzelleca, J.; Dewey, W. L. Narcotic and diabetes. II. Streptozotocin-induced diabetes selectively alters the potency of narcotic analgesics. Mechanism of diabetes: Morphine interaction. J. Pharmacol. Exp. Ther. 218:324–329; 1981.
33. Simon, G. S.;Dewey, W. L. Narcotic and diabetes. The effects of streptozotocin-induced diabetes on the antinociceptive potency of morphine. J. Pharmacol. Exp. Ther. 218:318–323; 1981.
34. Takano, Y.; Yaksh, T. L. Characterization of the pharmacology of intrathecally administered α_2-agonists and antagonists in rats. J. Pharmacol. Exp. Ther. 261:764–772; 1992.
35. Unnerstall, J. R.; Kopajtic, T. A.; Kuhar, M. J. Distribution of α_2-agonist-binding sites in the rat and human CNS: Analysis of some functional anatomic correlates of the pharmacological effects of clonidine and related adrenergic agents. Brain Res. Rev. 319:69–101; 1984.
36. Yaksh, T. L. Pharmacology of spinal adrenergic systems which modulate spinal nociceptive processing. Pharmacol. Biochem. Behav. 22:845–858; 1985.
37. Yaksh, T. L.; Rudy, T. A. Chronic catheterization of the spinal subarachnoid space. Physiol. Behav. 17:1031–1036; 1976.
38. Zeigler, D.; Lynch, S. A.; Muir, J.; Benjamin, J.; Max, M. B. Transdermal clonidine vs. placebo in painful diabetic neuropathy. Pain 48:403–408; 1992.
39. Zhang, P. L.; Patel, K. P. Blunted diuretic and natriuretic responses to central administration of clonidine in streptozotocin-induced diabetic rats. Diabetes 40:338–343; 1991.