

# Tolerance to $\beta,\beta'$ -Iminodipropionitrile (IDPN)-induced Neurobehavioural and Vestibular Toxicity in Diabetic Rats

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Key words: iminodipropionitrile; diabetes; neurotoxicity; dyskinesia; movement disorders; vestibular toxicity.

The present investigation was undertaken to study the neurotoxic effects of  $\beta,\beta'$ -iminodipropionitrile (IDPN) in normal, diabetic and insulin-treated diabetic rats. Sprague-Dawley male rats were divided into five groups: control, IDPN, diabetes, diabetes plus IDPN and diabetes plus insulin plus IDPN. The diabetes was induced with a single i.p. injection of streptozotocin ( $50 \text{ mg kg}^{-1}$ ). One month after the induction of diabetes, the rats were treated with IDPN ( $100 \text{ mg kg}^{-1}$ , i.p.) daily for 11 days. One of the diabetic groups treated with IDPN also received daily injection of insulin ( $25 \text{ U kg}^{-1}$ , s.c.), 1 h before IDPN. The rats were observed daily for abnormal head movements and circling. The grip strength of the forelimbs was also measured. In the IDPN group the dyskinetic symptoms appeared on the 8th day, whereas the onset of dyskinesia was on the 12th day in IDPN-treated diabetic rats. The incidence and severity of dyskinesia were significantly higher in IDPN-treated normal (non-diabetic) rats as compared to IDPN-treated diabetic rats. The treatment of diabetic rats with insulin normalized striatal dopamine (DA) turnover but partially reversed diabetes-induced protection against IDPN dyskinesia. There was severe degeneration of sensory hair cells in crista ampullaris of IDPN-treated normal rats, whereas the diabetic rats showed significant protection against IDPN-induced vestibular hair cell degeneration. In conclusion, our study clearly demonstrates that diabetic rats are resistant to IDPN-induced neurobehavioural and vestibular toxicity. The results also show that diabetes-induced protection against IDPN-induced dyskinesia can be partially reversed by insulin. The mechanism behind the decreased vulnerability of diabetic animals to IDPN remains to be resolved. Further studies are warranted to investigate this paradoxical phenomenon. Copyright © 1999 John Wiley & Sons, Ltd.

## INTRODUCTION

Iminodipropionitrile (IDPN) is a neurotoxin that has been shown to produce permanent movement disorder in rats and mice, characterized by repetitive head movements, circling, hyperactivity, retropulsion and swimming deficits.<sup>1,2</sup> This movement disorder was designated as ECC syndrome (excitation with choreiform and circling movements) by Selye<sup>3</sup> and later on as waltzing syndrome by Chou and Hartman.<sup>4</sup> The mechanism of IDPN-induced behavioural syndrome is not fully understood. Iminodipropionitrile has been shown to produce proximal axonal swelling and ballooning with neurofilamentous accumulation,<sup>4,5</sup> degeneration of distal elements<sup>6</sup> and deficits in conduction velocity and slow axonal transport.<sup>7,8</sup> However, the reversible nature of these pathological changes<sup>9,10</sup> is in contrast to the life-long permanence of the IDPN syndrome.<sup>11</sup> Moreover, chronic exposure to low doses of IDPN resulted in axonal pathology with no behavioural alterations.<sup>5</sup> Recently, Llorens and Rodriguez<sup>12</sup> have suggested that the vestibular toxicity, and not the axonopathy, is responsible for IDPN-induced behav-

our abnormalities. Iminodipropionitrile has been shown to produce time- and dose-dependent degeneration of vestibular sensory hair cells, leading to the development of ECC syndrome.<sup>13,14</sup> Similar behavioural deficits were observed in bilabyrinthectomized rats with no IDPN treatment.<sup>13</sup> An alternative hypothesis to explain IDPN-induced ECC syndrome is based on its ability to alter various central nervous system (CNS) neurotransmitters and neuromodulators.<sup>15–20</sup> The role of oxygen-derived free radicals in IDPN-induced neurodegeneration has also been proposed by Lohr *et al.*<sup>21</sup> Iminodipropionitrile-induced behavioural syndrome has been shown to be significantly attenuated by antioxidants.<sup>21,22</sup>

Diabetes mellitus is a metabolic disorder that affects various organ systems, including the CNS<sup>23,24</sup> and the peripheral nervous system.<sup>25,26</sup> Diabetes has also been shown to produce vestibular pathology and functional disturbances in the inner ear.<sup>27–29</sup> Alterations in several neurotransmitter systems<sup>30–33</sup> and increased oxidative stress<sup>34,35</sup> have also been reported in diabetic animals. The present investigation was undertaken to study the effect of vestibular and neurobehavioural toxicity of IDPN in streptozotocin (STZ)-induced diabetes in rats.

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## MATERIALS AND METHODS

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### Animal treatment

Male Sprague-Dawley rats (350–400 g) grown in our animal breeding facility were used. The animals were housed in a temperature-controlled room (24°C) with a 12-h light/dark cycle. Standard laboratory food and water were available *ad libitum* throughout the study. The rats of matching weights were randomly divided into five groups of 10 animals each. Three groups of rats were rendered diabetic with a single i.p. injection of STZ (50 mg kg<sup>-1</sup>) dissolved in normal saline, whereas the other two groups received saline only. After 30 days, all the animals were tested for blood glucose levels. The blood was taken via a tail nick, and the glucose concentration was determined with a glucometer (Accutrend, Germany). All the rats treated with STZ were found to be hyperglycaemic (blood glucose >445 mg dl<sup>-1</sup>). One group of the non-diabetic and two groups of the diabetic rats were treated with IDPN (100 mg kg<sup>-1</sup>, i.p.) daily (between 10.30 a.m. and 12.30 p.m.) for 11 days. Iminodipropionitrile was started 30 days after the single injection of STZ. One of the diabetic groups treated with IDPN also received 25 U kg<sup>-1</sup> of insulin (Humulin L, zinc suspension, from Eli Lilly & Co., USA) s.c. daily 1 h before IDPN administration for 11 days (during IDPN treatment only). The blood glucose level of all the rats was also monitored on the last day of study. The experimental protocol was approved by the Research and Ethics Committee of RKH, Saudi Arabia and was conducted in accordance with good laboratory practices.

### Behavioural studies

The dyskinetic behaviour was assessed by placing the rats individually in an open field arena, using the procedure of Diamond *et al.*<sup>36</sup> with some modifications.<sup>19,21</sup> The presence of dyskinetic head movements and circling was carefully monitored and the intensity of each symptom was counted for a period of 5 min. Forelimb grip strength was measured using a strain gauge attached to a wire mesh, as described by Moser and Boyes.<sup>37</sup> The animals were watched carefully, in a blinded fashion, for any behavioural abnormalities before the daily administration of the drugs. The complete behavioural studies were started when a clear indication of dyskinesia was observed in at least one animal from any treatment group (day 8 from the start of IDPN). The behavioural changes were recorded daily from day 8 to day 12, between 7.30 a.m. and 10.30 a.m.

### Analysis of striatal DA and DOPAC

The analysis of dopamine (DA) and 3,4-dihydroxyphenylacetic acid (DOPAC) in the striatum was done according to the procedure of Patrick *et al.*<sup>38</sup> The striata were weighed and homogenized for 10 s in 0.1 M perchloric acid containing 0.05% EDTA, using Teflon homogenizer. The homogenates were centrifuged immediately at 10 000 rpm and 4°C for 10 min. The supernatants were filtered using 0.45 µm pore filters

and analysed by high-performance liquid chromatography (HPLC). The HPLC system consisted of an electrochemical detector (Model 65, Metrohm, Herisani, Switzerland), an injector (Model 712, Waters Associate Inc. Milford, USA), a solvent delivery pump (Waters Model 510) and an integrator (Waters Model 745). The mobile phase consisted of a mixture of 0.1 M citric acid monohydrate, 0.1 M sodium acetate, 70% methanol, 100 µM EDTA and 0.01% sodium octane sulphonic acid, and the column was C-18 µBondapak (3.9 × 150 mm). The flow rate was maintained at 1 ml min<sup>-1</sup> and the injection volume was 200 µl.

### Inner ear histology

Rats were anaesthetized with diethyl ether and perfused (intracardiac) with 2.5% glutaraldehyde in 0.2 M phosphate buffer (pH 7.4). Temporal bones were removed and post-fixed in 10% neutral buffered formalin for 15 h. The bony labyrinth was decalcified by placing it in a decalcifying agent, Cal-Ex (Fisher Scientific, USA), for 48 h. The specimens were then processed overnight for dehydration, with increasing concentrations of alcohol and clearing with acetone and chloroform using an automatic processor (Shandon Southern 2L Processor MkII, UK). The specimens were embedded in paraffin blocks and sections of 5 µm thickness were stained with 1% toluidine blue for light microscopy observations.

### Statistics

The onset of dyskinesia was evaluated by the  $\chi^2$  test. Dunnett's multiple comparison test was used to determine the significance between the intensity of behavioural symptoms, grip strength data and striatal DOPAC/DA levels;  $P < 0.05$  was considered statistically significant.

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## RESULTS

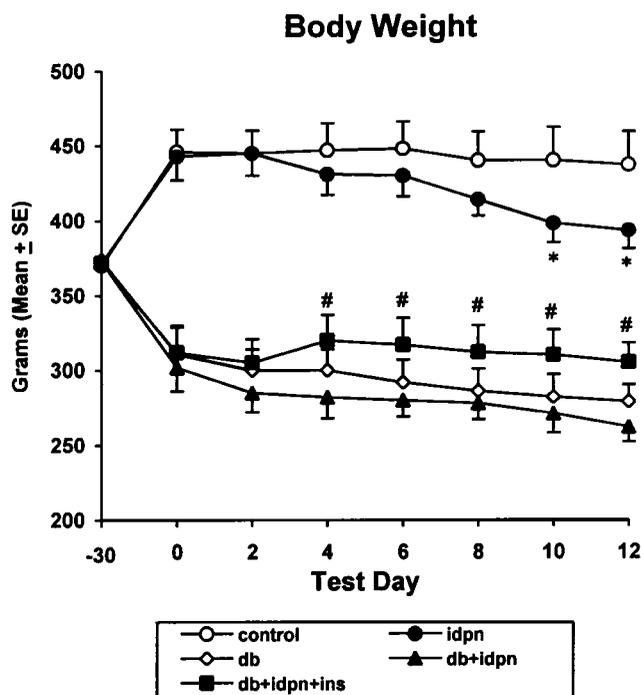
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### Animal body weight

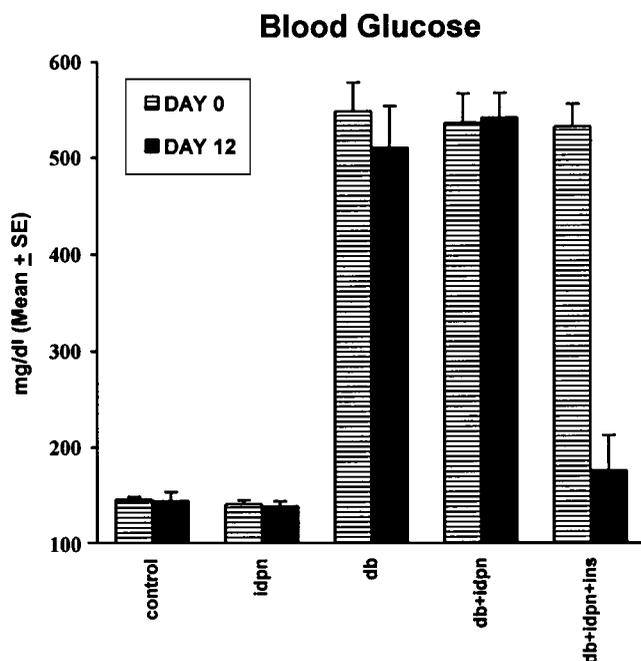
Diabetes of 1 month' duration caused a significant reduction (30%) in animal body weight gain compared to control rats. Administration of IDPN also significantly reduced (9.5%,  $P < 0.05$ ) the animal body weight in non-diabetic rats, whereas it caused a slight reduction (3.9%) in the body weight of diabetic animals. Insulin treatment showed a protective effect against diabetes-induced loss of body weight in the animals (Fig. 1).

### Blood glucose

The blood glucose level was  $141.66 \pm 7.20$  mg dl<sup>-1</sup> for the non-diabetic rats and  $525.24 \pm 34.20$  mg dl<sup>-1</sup> for the diabetic rats. Administration of insulin normalized the blood glucose level ( $174.78 \pm 36.54$ ) in diabetic rats (Fig. 2). The blood glucose level was not affected by IDPN treatment.



**Figure 1.** Effect of different treatments on animal body weight gain during the entire course of study. Day 0 (1 month after the induction of diabetes) was the start day of IDPN and insulin. \* $P < 0.05$  vs normal control and # $P < 0.05$  vs db + idpn group, Dunnett's test. Abbreviations: db, diabetes; ins, insulin; idpn, iminodipropionitrile; SE, standard error.



**Figure 2.** Blood glucose level in the rats in different treatment groups, monitored on day 0 (before starting ins/idpn) and on day 12 of study. For abbreviations, see Legend to Fig. 1.

### Onset and incidence of dyskinesia

The first appearance of dyskinetic behaviour was observed on day 8 in IDPN-treated non-diabetic rats, whereas IDPN dyskinesia appeared on the 12th day in

diabetic animals (Fig. 3). The incidence of dyskinesia was also significantly higher in non-diabetic rats as compared to diabetic animals following IDPN treatment. The administration of insulin partially reversed the diabetes-induced protective effects in IDPN-treated rats.

### Severity of dyskinesia

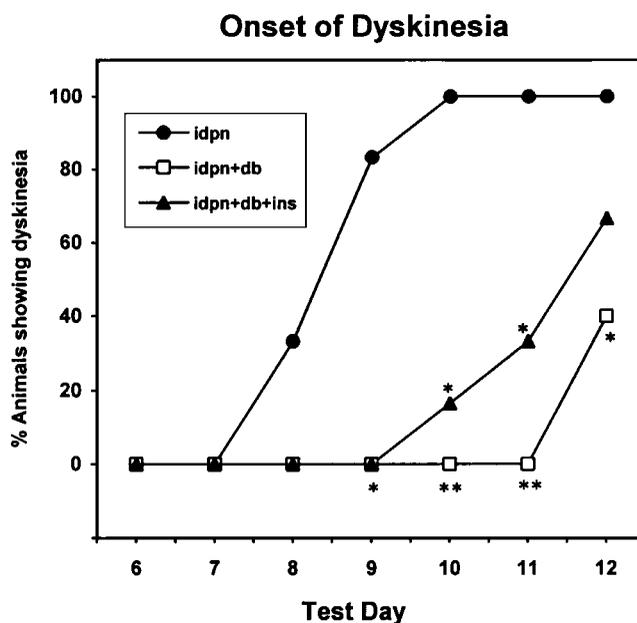
The intensity of dyskinetic behaviour, including abnormal head bobs and circling, was significantly higher in non-diabetic rats as compared to diabetic animals following IDPN treatment (Fig. 4). The insulin treatment reversed the diabetes-induced protection of IDPN dyskinesia; however, this effect was not found to be statistically significant. There was no dyskinetic abnormality in diabetic rats without IDPN treatment (Fig. 4).

### Grip strength

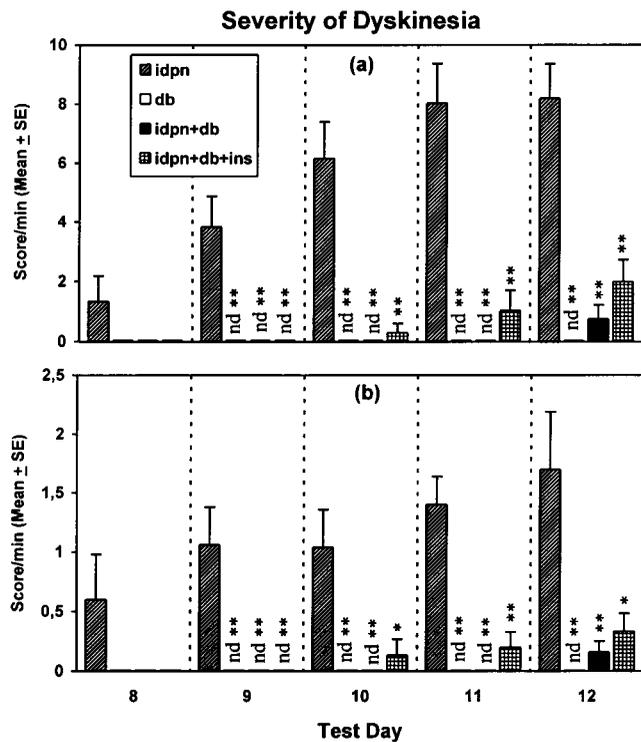
The administration of IDPN decreased the grip strength of the non-diabetic rats, which was statistically significant on days 10, 11 and 12. In diabetic rats, IDPN produced only a slight decrease in the grip strength, which was not significantly different from the diabetic control on any day of the behavioural studies (Fig. 5). The protective effect of diabetes on the IDPN-induced deficiency of grip strength was attenuated by insulin.

### Striatal DA turnover

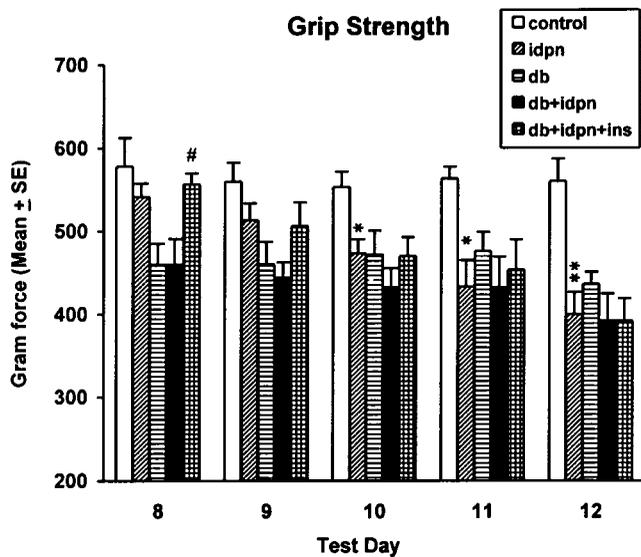
Administration of IDPN in normal rats insignificantly increased the striatal DA turnover (DOPAC/DA), whereas a decrease in DA turnover was observed in diabetic animals (Fig. 6). Insulin treatment reversed the effect of hyperglycaemia on DA turnover.



**Figure 3.** Onset of IDPN-induced dyskinesia in non-diabetic, diabetic and insulin-treated diabetic rats. \* $P < 0.01$  and \*\* $P < 0.001$  vs idpn alone group using the  $\chi^2$  test. For abbreviations, see Legend to Fig. 1.



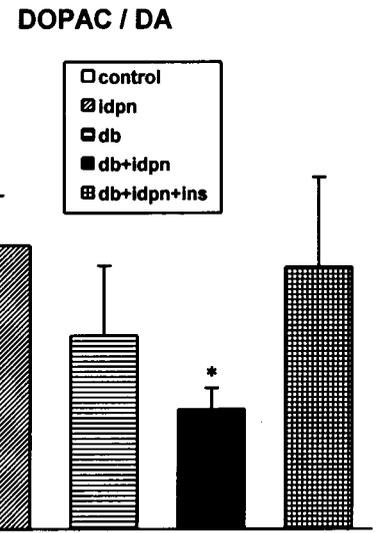
**Figure 4.** Severity scores of (a) abnormal head movements and (b) circling in different treatment groups. \* $P < 0.05$  and \*\* $P < 0.01$  vs idpn alone group using dunnett's multiple comparison test; nd = not detected. For abbreviations, see Legend to Fig. 1.



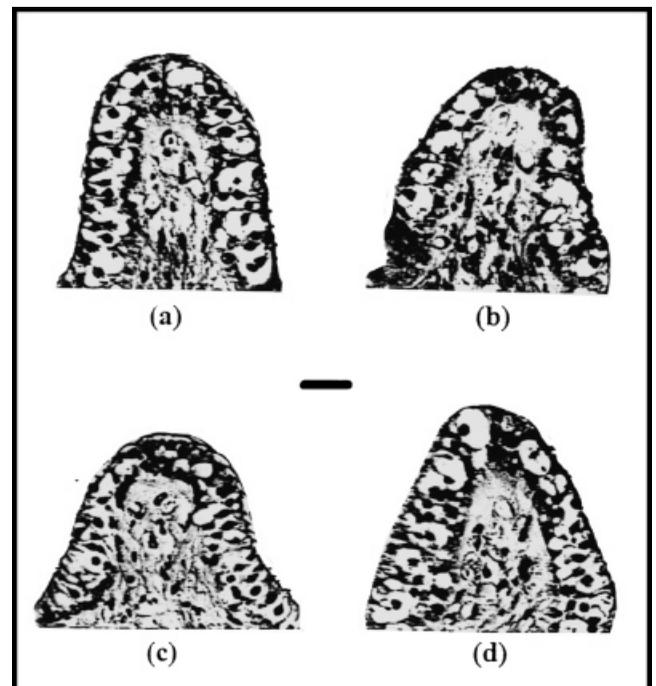
**Figure 5.** Effect of different treatments on forelimb grip strength. \* $P < 0.05$  and \*\* $P < 0.01$  vs normal control and # $P < 0.05$  vs diabetic control using Dunnett's test. For abbreviations, see legend to Fig. 1.

**Histological observation**

The crista ampullaris of the non-diabetic rats treated with IDPN showed severe degeneration of vestibular sensory hair cells (Fig. 7). The diabetic rats showed a significant protection against the toxic effect of IDPN on sensory hair cells. However, there was no significant change in the sensory epithelium of the diabetic and non-diabetic rats without IDPN treatment.



**Figure 6.** Effect of different treatments on striatal dopamine turnover. \* $P < 0.05$  vs idpn alone group using Dunnett's test. For abbreviations, see legend to Fig. 1.



**Figure 7.** Effect of IDPN on the degeneration of sensory hair cells in the crista ampullaris of non-diabetic and diabetic rats: (a) non-diabetic control; (b) IDPN alone; (c) diabetic control; (d) diabetes + IDPN. Administration of IDPN caused severe degeneration of sensory hair cells in the crista of non-diabetic rats, whereas such degeneration was not observed in the sensory epithelium of IDPN-treated diabetic rats. Scale bar = 20  $\mu$ m.

**DISCUSSION**

The results of this study clearly show that diabetic rats are resistant to IDPN-induced behavioural toxicity. There was a delay in the onset and a reduction in the incidence and severity of IDPN dyskinesia in diabetic rats compared to non-diabetic rats (Figs 3 and 4). Diabetic rats were also resistant to the IDPN-induced

deficiency in grip strength (Fig. 5). The administration of insulin partially reversed the diabetes-induced protection against IDPN dyskinesia. Administration of IDPN significantly reduced the body weight gain in non-diabetic rats on days 10 and 12. Earlier studies from our laboratory<sup>39</sup> and by other investigators<sup>37,40,41</sup> have shown that IDPN affects the body weight during the treatment period only, after which the weight gain of IDPN-treated and control animals showed a similar pattern. Our histopathological studies showed a severe degeneration of sensory hair cells in the crista ampullaris of IDPN-treated non-diabetic rats. Although diabetes alone did not produce any change in sensory epithelium, it showed significant protection against IDPN-induced pathological changes (Fig. 7). Both IDPN<sup>12-14</sup> and diabetes<sup>27,28</sup> have been shown to cause vestibular toxicity but they produce pathological changes at different sites in the vestibular system; whereas the saccule is more susceptible to the pathology of diabetes,<sup>28</sup> the crista and utricle are mainly affected by IDPN.<sup>14</sup>

The mechanism accounting for diabetes-induced protection against IDPN-induced neurobehavioural and vestibular toxicity is far from clear. The results of our biochemical studies showed an increase in striatal DA turnover in IDPN-treated non-diabetic rats, whereas a significant reduction of DA turnover was observed in diabetic rats (Fig. 6). Increased turnover of striatal DA by IDPN has been reported earlier<sup>18</sup> and DA antagonists have been shown to protect rats against IDPN-induced neurobehavioural toxicity.<sup>15</sup> On the other hand, an inverse relationship between hyperglycaemia and dopaminergic activity has been observed<sup>31,42,43</sup> and the behavioural effects of DA agonists were found to be diminished in diabetic rats.<sup>44,45</sup> Insulin does not affect DA levels in normal rats<sup>46</sup> but it significantly protects animals against diabetes-induced changes in DA turnover.<sup>30</sup> Although the administration of insulin to IDPN-treated diabetic rats normalized the DA turnover (Fig. 6), it failed to reverse significantly the diabetes-induced attenuation of IDPN dyskinesia (Fig. 4).

Earlier studies from our laboratory suggest that IDPN-induced neurotoxicity is accompanied by ischaemic changes in the brain, and increasing the levels of endogenous vasodilators and natural neuroprotectant adenosine by dipyrindamole significantly attenuated IDPN-induced neurotoxicity.<sup>19</sup> Diabetes has been

shown to protect nerve<sup>47,48</sup> and muscle<sup>49</sup> tissue against ischaemic damage. The protective effect of diabetes against ischaemia has been attributed to the abundance of energy substrates that can be utilized by the tissue during anaerobic conditions.<sup>49,50</sup> *In vitro* studies on isolated mammalian nerve also showed a better tolerance to anoxia in a medium containing high glucose concentrations.<sup>51</sup> The role of oxygen-derived free radicals in IDPN-induced neurotoxicity has also been suggested by several investigators.<sup>19,21,22</sup> Sagone *et al.*<sup>52</sup> have reported the free radical scavenging property of glucose. Thus, the diminished toxicity of IDPN in diabetic rats may also be partially attributed to the antioxidant effect of glucose against IDPN induced oxidative stress. Furthermore, the altered bioavailability of IDPN due to impaired absorption, metabolism and excretion has been shown to affect its toxicity.<sup>41,53-55</sup> Recent studies show that diabetes may interfere with the bioavailability of xenobiotics, including gentamicin,<sup>56,57</sup> cisplatin,<sup>58,59</sup> cephaloridine<sup>60</sup> and cadmium-metallothionein,<sup>61</sup> resulting in reduced access of these drugs to the target sites. Sarangarajan and Cacini<sup>59</sup> observed a 50% reduction in the bioavailability of cisplatin (administered intraperitoneally) in diabetic rats compared to non-diabetic animals, suggesting diabetes-related changes in the peritoneal membrane or its blood supply. An enhanced glomerular filtration rate in diabetes<sup>62,63</sup> may also contribute to rapid clearing of IDPN or its toxic metabolites. However, further studies on the bioavailability of IDPN in diabetic rats are suggested.

In conclusion, our study clearly demonstrates that diabetic rats are resistant to IDPN-induced neurobehavioural and vestibular toxicity. The results also show that diabetes-induced protection against IDPN-induced dyskinesia can be partially reversed by insulin. The mechanism behind the decreased vulnerability of diabetic animals to IDPN remains to be resolved. Further studies are warranted to investigate this paradoxical phenomenon.

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