Water intake disorder in a DEND syndrome afflicted patient with R50P mutation

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Abstract. In this study, we present a case of developmental delay, epilepsy and neonatal diabetes (DEND) syndrome in a young male patient with the R50P mutation located in the Kir6.2 subunit of the ATP-sensitive K+ (KATP) channel. Whereas most patients with DEND syndrome are resistant to sulfonylurea therapy, our patient was responsive to sulfonylurea, lacked the most common neurological symptoms, such as epilepsy, but refused to drink water. His serum electrolytes and plasma osmolarity were normal but the serum vasopressin level was increased. To investigate the underlying mechanism of his water intake disorder, a 5 μL aliquot of 340 μM KATP channel opener diazoxide or 100 μM KATP channel inhibitor glibenclamide was injected into the third ventricle of the rat brain, and water intake was monitored. Although the injection of glibenclamide had no effect, injection of diazoxide significantly increased water intake by about 1.5 fold without affecting food intake. This result indicates that the KATP channel activity in the brain may have an influence on water intake. Here, we present the first case of a DEND syndrome–afflicted patient with water intake disorder and increased serum vasopressin level, possibly related to altered KATP channel activity.

Key words: KATP channel, DEND syndrome, Sulfonylurea

THE ATP-SENSITIVE K+ (KATP) channel is a key factor that couples cell metabolisms with the changes in cell excitability in various tissues including pancreatic β-cells and the brain [1, 2]. It is a hetero-octameric complex composed of four pore-forming Kir6.2 subunits and four regulatory sulfonylurea receptor 1 (SUR1) subunits. In pancreatic β-cells, KATP channels play a major role in regulating insulin secretion. A rise in plasma glucose stimulates glucose uptake and metabolism, causing an increase in intracellular ATP in β-cells. These changes in adenine nucleotide concentration result in the closure of the KATP channel. The decrease in the membrane’s K+ permeability by closure of the KATP channel depolarizes the cell membrane and allows the voltage-dependent Ca2+ channel (VDCC) to open. The influx of Ca2+ into the cell cytoplasm through VDCC causes an increase in [Ca2+]i, and this increase triggers the release of insulin [3].

Mutations in KCNJ11, the gene encoding the Kir6.2 subunit, are known to cause neonatal diabetes. Some mutations are known to give rise to a severe form of disease, the DEND syndrome [3, 4]. Aside from hyperglycaemia, DEND syndrome is characterized by severe neurological features such as developmental delay, epilepsy, and muscle weakness [5].

The case we present in this paper was diagnosed with DEND syndrome at the age of 7 months due to the presence in his genome of a mutation of arginine to proline in residue 50 of Kir6.2 (R50P). Previously, we reported that the R50P mutation of Kir6.2 causes DEND syndrome [6]. A functional electrophysiological study on the KATP channel with the R50P mutation
suggested that the K<sub>ATP</sub> channel inhibitor sulfonylureas may be effective for treating both hyperglycaemia and neurological symptoms of patients exhibiting the R50P DEND syndrome [6]. Based on these findings, our subject underwent sulfonylurea therapy (glibenclamide) at the time of diagnosis. Although the patient still showed severe developmental delay, his epilepsy ceased soon after the initiation of glibenclamide therapy. Moreover, he started walking unassisted. Thus, the development of his syndrome is in stark contrast with other patients having DEND syndrome–causing mutations. However, the patient started to show signs of resisting water intake from the time he became able to consume food unassisted. His serum electrolytes and osmolarity was normal but his serum vasopressin level was increased. It is possible that water intake disorder is related to the alteration of K<sub>ATP</sub> channel activity in the brain.

However, to date, no reports have been published indicating the existence of a link between the K<sub>ATP</sub> channel and the regulation of water intake. In order to investigate the relationship between water intake disorder and K<sub>ATP</sub> channel mutation in our patient, we injected the opener and inhibitor of the K<sub>ATP</sub> channel into the third ventricle (3V) of a rat brain and investigated whether pharmacological intervention into the activity of the K<sub>ATP</sub> channel may affect water intake in the rodent. In this experiment, we observed an increase in water intake for 1 hour after injection of K<sub>ATP</sub> channel opener, diazoxide without affecting the volume of food intake, indicating the possible link between the K<sub>ATP</sub> channel and water intake regulation. Here, we report a case of a young male with DEND syndrome presenting a water intake disorder with increased serum vasopressin level possibly in relation to alteration of K<sub>ATP</sub> channel activity.

**Case Report**

The subject in this study, a 7-year-old male, developed symptoms of hyperglycaemia and epilepsy with brain wave pattern of periodic hypersynchrony at 4 months after birth. The patient’s muscle was hypotonic and his head and neck was not stable at this stage. At this time, his HbA1c was 10.2%. Test for islet cell autoantibodies against glutamic acid decarboxylase (GAD), insulinoma-associated antigen and insulin were negative. Once insulin injection therapy began, the subject’s HbA1c was decreased to 8.5%. At 7 months after birth, he was diagnosed with DEND syndrome with an R50P mutation in Kir6.2. Sulfonylurea treatment using glibenclamide (0.8 mg/kg/day) was initiated upon diagnosis. Following treatment, no epilepsy was observed, blood glucose was normalized, and HbA1c decreased to 6.5%. Insulin injections were stopped completely 3 weeks after starting glibenclamide therapy. At present, the dose of glibenclamide is increased to 1.2 mg/kg/day, and blood glucose has remained stable at 6.5% in HbA1c. Regarding the patient’s psychomotor development, although his muscle is hypotonic, he became able to independently stand, walk, and perform simple everyday tasks (such as eating). By age of 4 years old, the patient started to become emotionally unstable. At this point, patient started to show signs of resisting water intake. Initially, it was considered to be due to the developmental delay. However, gradually it became evident that patient is not taking water unless he is forced to do so by his parents. At age of seven, he is still resisting to take water. Although urine sample analysis was not performed since collection of his urine was impossible due to the developmental delay, his laboratory data of serum electrolytes and osmolarity showed no sign of dehydration (Table 1). However, the serum vasopressin level was found to be increased to almost twice as much as normal level (normal value: less than 4.2pg/mL) (Table 1).

**Functional Study**

A 26-gauge guide cannula was placed into the third ventricle (3V) of a male Wistar rat brain, as previously described [7]. Then, a 5-μL aliquot of 340 μM K<sub>ATP</sub> channel opener diazoxide or saline was injected. Subsequent cumulative water and food intake were monitored.

As shown in Fig. 1, the 3V injection of diazoxide increased water intake; the mean values of water intake for 1 hour post-injection were 2.3 ± 0.5 g (n =
Water intake disorder in DEND syndrome

Most of the mutations that cause neonatal diabetes alone present reduced ATP sensitivity but do not change the intrinsic open probability (P_o) of the KATP channel. However, most mutations that cause DEND syndrome increase the P_o of the KATP channel. This increase in P_o results in less effectiveness of both ATP and sulfonylureas as blockers. Our previous paper reported that the R50P mutation does not alter the P_o of the mutated KATP channel [6]. This may be explained by the location of Arg50 in Kir6.2. Based on the structure of the human Kir6.2 subunit shown in Fig. 2 (cover range of 31–356; modelled using Swiss model program with the template structure of Kir6.2, PDB ID 3SPG) [10–13], Arg50 residues lay far away from the channel pore of Kir6.2. Most DEND syndrome mutations with increased P_o are located around the channel pore area. Also, it was predicted in our previous paper that sulfonylurea treatment may be effective for the patient with R50P mutation [6]. Consistent with these findings, our subject with the R50P mutation responded successfully to sulfonylurea therapy, showing less severe neurological disorders such as epilepsy.

Discussion

Sulfonylurea treatment is effective in most patients with KCNJ11 mutations that cause neonatal diabetes alone. However, sulfonylureas are far less effective in patients with mutations that cause DEND syndrome [8, 9]. This can be explained by the property of the mutation that alters the single-channel kinetics of the KATP channel. Most of the mutations that cause neonatal diabetes alone present reduced ATP sensitivity but do not change the intrinsic open probability (P_o) of the KATP channel. However, most mutations that cause DEND syndrome increase the P_o of the KATP channel. This increase in P_o results in less effectiveness of both ATP and sulfonylureas as blockers. Our previous paper reported that the R50P mutation does not alter the P_o of the mutated KATP channel [6]. This may be explained by the location of Arg50 in Kir6.2. Based on the structure of the human Kir6.2 subunit shown in Fig. 2 (cover range of 31–356; modelled using Swiss model program with the template structure of Kir6.2, PDB ID 3SPG) [10–13], Arg50 residues lay far away from the channel pore of Kir6.2. Most DEND syndrome mutations with increased P_o are located around the channel pore area. Also, it was predicted in our previous paper that sulfonylurea treatment may be effective for the patient with R50P mutation [6]. Consistent with these findings, our subject with the R50P mutation responded successfully to sulfonylurea therapy, showing less severe neurological disorders such as epilepsy.

Fig. 1 Cumulative water intake after 3V injection of diazoxide. Open bar, control group (n = 5); closed bar, diazoxide injected group (n = 5). The data are presented as means ± SE. Statistical significance was evaluated by unpaired t test. P values <0.05 were considered statistically significant.

Fig. 2 Location of Arg50 in a tetrameric Kir6.2 model. Arg50 is shown as sticks (red). Each subunit is shown in a different color.
and muscle weakness than usually observed in patients with DEND syndrome. However, our subject started to develop sign of water intake disorder after becoming capable of independent food and liquid consumption. To date, there are no reports of water intake disorder in patients with DEND syndrome, and our study describes the first subject to show such a symptom. It is unclear whether water intake disorder is general symptom of DEND syndrome or unique only to our case with R50P mutation. However, there is a possibility that water intake disorder presented in our subject is perhaps not entirely unique for R50P mutation. It should be taken into account that most patients with DEND syndrome exhibit more severe symptoms than those observed in our subject and require assistance when ingesting meals and liquids [14, 15]. Such patients are unlikely to show signs of water intake disorder. On the other hand, our subject responded to sulfonylurea therapy with considerable success and, consequently, could ingest food and liquid without assistance and therefore became able to resist water intake based on his own will. However, in order to clarify whether water intake disorder is general symptom of DEND syndrome or unique to our case, further careful observation of other DEND syndrome patients is required.

The underlying mechanism of water intake disorder in our case is not clear. The K\text{ATP} channel is known to be expressed in various areas in the brain, including the hippocampus, substantia nigra, and hypothalamus [16, 17]. The roles of the K\text{ATP} channel in the brain include central regulation of food intake. K\text{ATP} channel is a necessary component of glucosensing in glucose-excited neurons in brain areas involved in food intake regulations [18]. K\text{ATP} channel is also present in other neurons that have no glucose sensing capability [19]. In these neurons K\text{ATP} channel is considered to be closed in normal state but play neuroprotective role by opening and hyperpolarizing the neuron against over-release of glutamate during cerebral ischemia and other severe brain stress [20].

One possible mechanism of our patient’s water intake disorder is direct involvement of K\text{ATP} channel in water intake regulation in brain. In this study, we showed that the injection of the K\text{ATP} channel opener diazoxide into the 3V of the brain induces an increase of water intake. In addition, we also found that the effect of diazoxide on increasing water intake lasted for 1 hour but not 2 hours post-injection into the brain. Earlier reports show that the effect of diazoxide on opening the K\text{ATP} channel is reversible and can easily be washed out [21–24]. This supports the concept that the temporal increase of water intake after injection of diazoxide into rat brain is mediated by the diazoxide-induced K\text{ATP} channel activation of brain. However, our present study may not explain the condition of our case, as the patient showed reduction of water intake, not increase. Majority of K\text{ATP} channel in brain, which function as neuroprotection, is considered to be closed in normal state in healthy subject. It is possible to speculate that, in our subject these normally closed K\text{ATP} channel in brain may have been opened due to the R50P mutation and glibenclamide treatment induced closure of these pathologically opened brain K\text{ATP} channel. This may have contributed to the development of water intake disorder. As sulfonylureas are prescribed worldwide for the treatment of type 2 diabetes, it may be argued that there are as of yet no reports of water intake disorder from sulfonylurea-treated diabetic patients. Furthermore, there are no reports of water intake disorders from hyperinsulinemia patients with diazoxide treatment. This may be explained by the fact that K\text{ATP} channel in brain of type 2 diabetic patient is genetically unaffected and therefore majority are closed even without glibenclamide. Also, the dose of sulfonylureas used for the treatment of DEND syndrome is higher than dose used to treat type 2 diabetes. It can also indicate the possibility that the dose of sulfonylureas used to treat type 2 diabetes may not be high enough to cross the blood brain barrier and reach the central nervous system and cause any effect on brain K\text{ATP} channel. This may also be true for the dosage of diazoxide used to treat hyperinsulinemia. Therefore, patients with type 2 diabetes/hyperinsulinemia undergoing sulfonylurea/diazoxide treatment are unlikely to exhibit signs of water intake disorder.

Another possibility is through modulation of vasopressin secretion through K\text{ATP} channel. In the present case, the patient showed increase of serum vasopressin level. Vasopressin is related in water intake regulation and its containing neuron is located in supraoptic nucleus and K\text{ATP} channel is considered to be involved in its secretory mechanism [25, 26]. It is possible that modulation of K\text{ATP} channel activity by DEND syndrome mutation or sulfonylurea treatment may have affected vasopressin secretion and thereby affected water intake in our case.

Although our data indicate the possible link between altered K\text{ATP} channel activity and water intake disorder,
further studies of \( K_{ATP} \) channel functions and detail observation of other DEND syndrome patients are required to elucidate the underlying mechanisms in the conditions such as those in our subject.

In this paper, we show the first case of DEND syndrome patient with water intake disorder and increase of serum vasopressin level. Also, it is well known that one of the first signs in the course of developing type 2 diabetes is excessive thirst. Therefore, an early detection of disrupted water intake is important for prompt treatment of type 2 diabetes. Considering the increase of patients with type 2 diabetes worldwide and the fact that sulfonylurea drugs are widely used for the treatment of diabetes, our subject and the functional study presented in this paper may contain important information for understanding the pathophysiology of both DEND syndrome and type 2 diabetes.

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Disclosure

None of the authors have any potential conflicts of interest associated with this research.

References


