Dextromethorphan and Dextrorphan Influence Insulin Secretion by Interacting with $K_{\text{ATP}}$ and L-type Ca$^{2+}$ Channels in Pancreatic $\beta$-Cells

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ABSTRACT

The NMDA receptor antagonist dextromethorphan (DXM) and its metabolite dextrorphan (DXO) have been recommended for treatment of type 2 diabetes mellitus because of their beneficial effects on insulin secretion. This study investigates how different key points of the stimulus-secretion coupling in mouse islets and $\beta$-cells are influenced by DXM or DXO. Both compounds elevated insulin secretion, electrical activity, and $[\text{Ca}^{2+}]_\text{c}$ in islets at a concentration of 100 $\mu$M along with a stimulating glucose concentration. DXO and DXM increased insulin secretion approximately 30-fold at a stimulatory glucose concentration (3 mM). Patch-clamp experiments revealed that 100 $\mu$M DXM directly inhibited $K_{\text{ATP}}$ channels by about 70%. Of note, DXM (3 mM). Of note, DXM decreased the current through L-type Ca$^{2+}$ channels about 25%, leading to a transient reduction in Ca$^{2+}$ action potentials. This interaction might explain why elevating DXM to 500 $\mu$M drastically decreased insulin release. DXO inhibited $K_{\text{ATP}}$ channels almost equally. In islets of $K_{\text{ATP}}$ channel–deficient sulfonylurea receptor 1 knockout mice, the elevating effects of 100 $\mu$M DXM on $[\text{Ca}^{2+}]_\text{c}$ and insulin release were completely lost. By contrast, 100 $\mu$M DXO still increased glucose-stimulated insulin release around 60%. In summary, DXM-induced alterations in stimulus-secretion coupling of wild-type islets result from a direct block of $K_{\text{ATP}}$ channels and are partly counteracted by inhibition of L-type Ca$^{2+}$ channels. The stimulatory effect of DXO seems to be based on a combined antagonism on $K_{\text{ATP}}$ channels and NMDA receptors and already occurs under resting conditions. Consequently, both compounds seem not to be suitable candidates for treatment of type 2 diabetes mellitus.

SIGNIFICANCE STATEMENT

This study shows that the use of dextromethorphan as an antidiabetic drug can cause unpredictable alterations in insulin secretion by direct interaction with $K_{\text{ATP}}$ and L-type Ca$^{2+}$ channels besides its actual target, the NMDA receptor.

Introduction

Dextromethorphan (DXM) is known as a nonopioid over-the-counter cough suppressant. Currently, there are many clinical studies investigating the therapeutic potential of DXM mainly for treatment of diseases related to the central nervous system. Suggested indications are depression, traumatic brain injury, stroke, seizure, pain, methotrexate neurotoxicity, Parkinson disease, and autism (Nguyen et al., 2016). In 2010, the Food and Drug Administration approved the use of DXM in combination with quinidine for patients suffering from pseudobulbar affect. Apart from its neuroprotective properties, DXM has been suggested for treatment of type 2 diabetes mellitus (Marquard et al., 2015). These versatile effects are based on the antagonism of DXM at the NMDA receptor (NMDAR). The NMDAR is a voltage- and ligand-gated ion channel that opens selectively for cations (especially Na$^+$, K$^+$, and Ca$^{2+}$) upon activation. The heterotetrameric channel is activated by concurrent depolarization to release the Mg$^{2+}$ block out of the pore and by simultaneous binding of its ligand and a coactivator (e.g., glycine) (Vylicky et al., 2014; Zhu et al., 2016). In the 90s, it became evident that NMDARs exist in insulin-secreting $\beta$-cells and take part in the regulation of glucose-induced insulin secretion (Gonoi et al., 1994; Inagaki et al., 1995; Molnar et al., 1995). There are controversial results about the function of NMDARs. Inhibition of the NMDAR either results in increased insulin secretion (Marquard et al., 2015), in no alteration of insulin secretion (Bertrand et al., 1992; Chan et al., 1997; Patterson et al., 2016; Imai et al., 2018; Suwandhi et al., 2018), or in reduced insulin secretion (Patterson et al., 2016). The exact molecular mechanism is not clear yet, but it was demonstrated that NMDAR activity contributes to the trafficking of $K_{\text{ATP}}$ channels to the plasma membrane in INS-832/13 cells (Wu et al., 2017). It is conceivable that, as in subthalamic neurons, Ca$^{2+}$ influx through NMDA-gated channels activates $K_{\text{ATP}}$ currents through a nitric oxide/cGMP-dependent pathway (Shen and Johnson, 2010). Hence, NMDAR

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ABBREVIATIONS: a.u., arbitrary fluorescence unit; Ca, channel; voltage-regulated Ca$^{2+}$ channel; DXM, dextromethorphan; DXO, dextrorphan; FOPP, fraction of plateau phase; $K_{\text{ATP}}$, channel; ATP-dependent K$^+$ channel; $K_{\text{Ca}}$, channel, Ca$^{2+}$-regulated K$^+$ channel; Kir6.2, inwardly-rectifying K$^+$ channel subunit 6.2; K$_s$, channel, voltage-dependent K$^+$ channel; MEA, microelectrode array; NMDA, N-methyl-D-aspartate; NMDAR, NMDA receptor; SURI-KO, sulfonylurea receptor 1 knockout; $V_m$, membrane potential.
activation seems to act as a negative-feedback regulator for glucose-induced insulin secretion by promoting K⁺ outward currents, which initiate repolarization of the membrane potential. Marquard et al. recommended DXM, or rather its active metabolite dextromethorphan (DXO), for treatment of type 2 diabetes mellitus because of its beneficial effects on insulin secretion (Marquard et al., 2015, 2016). Direct effects on important steps of the stimulus-secretion coupling were accomplished with MK-801 (also known as dizocilpine), another noncompetitive NMDAR antagonist. MK-801 changed the pattern of glucose-induced Ca²⁺ oscillations in mouse islets, increased the plateau fraction of Ca²⁺ oscillations, and prolonged the characteristic bursts of electrical activity in glucose-stimulated β-cells (Marquard et al., 2015). However, there is evidence that MK-801 not only blocks NMDAR but also interacts with voltage-dependent K⁺ channels (Kᵥ channels). A direct inhibition of Kᵥ channels was shown in dissociated sympathetic neurons and mesenteric arterial smooth muscle cells of rats (Woolorton and Mathie, 1995; Kim et al., 2015). Several Kᵥ channel subunits that exist in vascular smooth muscle cells are expressed in insulin-secreting cells (MacDonald and Wheeler, 2003). As repolarization of β-cell action potentials is mediated by activation of Kᵥ channels (MacDonald and Wheeler, 2003), the abovementioned effects of MK-801 on Ca²⁺ oscillations might not only be based on interaction with the NMDAR. The aim of this work was to elucidate whether DXM and DXO, respectively, influence insulin secretion by affecting plasma membrane potential and cytosolic Ca²⁺ similar to MK-801. To further investigate whether the observed drug effects are attributable to inhibition of NMDAR and concomitant elimination of its negative feedback on the stimulus-secretion cascade or involve other mechanisms, it was tested whether the two compounds directly interact with Kₐ₅P and/or L-type Ca²⁺ channels of primary mouse β-cells.

Methods

Cell and Islet Preparation. Experiments were performed with islets of Langerhans from adult male and female C57BL/6N mice (Charles River, Sulzfeld, Germany). The principles of laboratory animal care were followed according to German laws (Az. 53.5.32.7.1/MS-12668, health and veterinary office Münster, Germany). Breeding pairs of sullfonylurea receptor 1 knockout (SUR1-KO) mice (on a C57BL/6 background) as well as SUR1-KO islets for some initial experiments were kindly provided by Prof. Dr. Gisela Drews (Institute of Pharmacology, Department of Pharmacology and Clinical Pharmacy, University of Tubingen, Germany). Mice were euthanized using CO₂ and pancreatic islets of both genotypes were isolated similarly by collagenase digestion. Murine islets were dispersed to single cells by trypsin-EDTA treatment (0.25%, 2 minutes). Islets or cells were cultured in RPMI 1640 medium (11.1 mM glucose) supplemented with 10% fetal calf serum, 100 μM penicillin, and 100 μg/ml streptomycin at 37°C in a 5% CO₂, humidified atmosphere.

Solutions and Chemicals. Electrophysiological measurements and recordings of [Ca²⁺] were performed with a bath solution of (in millimolars) 140 NaCl, 5 KCl, 1.2 MgCl₂, 2.5 CaCl₂, 10 HEPES, and glucose as indicated (pH 7.4, adjusted with NaOH). For determination of Ca²⁺ currents, the bath solution contained (in millimolars) 115 NaCl, 1.2 MgCl₂, 10 CaCl₂, 20 tetraethylammonium chloride, 10 HEPES, 15 glucose, and 0.1 tobutamide (pH 7.4, adjusted with NaOH). Pipette solution for recording of plasma membrane potential (Vₘ) consisted of (in millimolars) 10 KCl, 10 NaCl, 70 K₂SO₄, 4 MgCl₂, 2 CaCl₂, 10 EGTA, 5 HEPES, and 0.27 amphotericin B (pH 7.15, adjusted with KOH). For Ca²⁺ currents, the pipette solution was composed of (in millimolars) 50 CsCl, 70 N-methyl-d-glucamine, 58 HCl, 4 MgCl₂, 2 CaCl₂, 10 EGTA, 3 Na₂ATP, and 10 HEPES (pH 7.15, adjusted with CsOH). To determine Kₐ₅P currents, pipette solution was composed of (in millimolars) 130 KCl, 4 MgCl₂, 2 CaCl₂, 10 EGTA, 0.65 Na₂ATP, and 20 HEPES (pH 7.15, adjusted with KOH). Krebs-Ringer-HEPES solution (KRH) for insulin secretion was composed of (in millimolars) 122 NaCl, 4.7 KCl, 1.1 MgCl₂, 2.5 CaCl₂, 10 HEPES, glucose as indicated, and 0.5% bovine serum albumin (pH 7.4, adjusted with NaOH). Dextromethorphan hydrobromide was obtained from Alfa Aesar (Thermo Fisher, Karlsruhe, Germany); memantine hydrochloride from Fisher Scientific GmbH (Schwerte, Germany); Collagenase P from Roche Diagnostics (Mannheim, Germany); Corning Matrigel basement membrane preparation from VWR (Darmstadt, Germany); and RPMI 1640, fetal calf serum, and penicillin/streptomycin from Life Technologies (Darmstadt, Germany). Fura-2 acetoxyxymethyl ester and rat insulin were ordered from Biotrend (Köln, Germany). Dextromethorphan tartrate and all other chemicals were from Sigma-Aldrich (Taufkirchen, Germany) or Diagonal (Münster, Germany).

Insulin Secretion. After preparation, islets were kept overnight in RPMI medium. The next day, medium was removed, and islets were incubated in KRH with 5.6 mM glucose for 1 hour and thereafter in KRH buffer with 3 mM glucose for 0.5 hours. In the case of preincubation, the compounds were already added to the KRH buffer containing 5.6 and 3 mM glucose. For determination of insulin secretion, batches of five islets were incubated at 37°C for 60 minutes with the indicated substances and glucose concentrations. Each condition was prepared in triplicate. For analysis of the kinetics of insulin release, bath chambers were equipped with 50 islets and perfused with KRH at the indicated conditions at a rate of 0.7 ml/min at 37°C. Eluate samples were taken every 2 minutes. Insulin concentration was quantified by a radioimmunoassay using rat insulin as standard. In perfusion experiments, the substimulatory phase was evaluated by determining the area under the curve of a 15-minute time interval. For analysis of glucose-stimulated secretion, the area under the curve was calculated starting from the first rise in insulin release after changing the glucose concentration to 15 mM until the end of the experiment (approximately 50 minutes).

Measurement of [Ca²⁺]. For determination of [Ca²⁺], islets were cultured for 1 to 2 days on glass coverslips coiled with poly-1-lysine. Islets were loaded with fura-2 acetoxyxymethyl ester (5 μM, 37°C, 30 minutes) and thereafter perfused with bath solution plus indicated test substances. Fluorescence was excited at 340 and 380 nm, and emission was measured by a digital camera (filter 515 nm). Alterations in mean [Ca²⁺], were evaluated by calculating the average of the mean Ca²⁺ concentration in bath solution with 3 or 8 mM glucose for 15 minutes (1-hour preincubation experiments), for the last 5 minutes with the respective test compounds (acute application), or for the last 2 minutes in the presence of 0.5 mM glucose. The effect of DXM on the interburst duration was calculated by averaging the two preceding interburst intervals under control conditions in comparison with the period in which [Ca²⁺] remained at the basal niveau after addition of DXM. To confirm that the islets were metabolically intact, bath solution with 0.5 or 8 mM glucose was applied at the end of each experiment. Pancreatic β-cells exhibit characteristic glucose-evoked oscillations, which disappear in the presence of a low glucose concentration (Grapengiesser et al., 1988).

Electrophysiology. Patch-clamp experiments were performed with single islet cells. Pipettes were pulled from borosilicate glass capillaries (resistance of 3–5 MΩ). An EPC-10 patch-clamp amplifier (software “Patchmaster” and “Fitmaster”; HEKA, Germany) was used for data acquisition and analysis. Vₘ was measured in the current-clamp mode. Vₘ was evaluated by determination of the plateau potential (from which action potentials start) for a period of 1 minute before changing bath solution. The frequency of action potentials was evaluated during a 4- to 5-minute interval. Kₐ₅P current was recorded by application of 300-millisecond pulses to −80 and −60 mV, respectively, starting from a holding potential of −70 mV.
Results

DXM and DXO Increase Insulin Secretion. The influence of the NMDAR antagonists DXM and its metabolite DXO on insulin secretion was investigated in the presence of different glucose concentrations. Islets incubated with 100 μM DXM for 1 hour secreted significantly more insulin during stimulation with 3, 8, 10, or 15 mM glucose compared with control (Fig. 1A). The same effect was evoked by 100 μM DXO, but to achieve this, the incubation time had to be elevated from 1 to 2.5 hours. Most likely, this is due to the higher hydrophobicity of the demethylated compound (Yu and Haining, 2001). In these experiments, islets were already elevated from 1 to 2.5 hours. Most likely, this is due to the random number of islets attaching on an electrode of the microelectrode array.

Statistics. Data were collected from islets or islet cells of at least three independent mouse preparations for each series of experiments. Values are given as scatter plots and means ± S.D. Data were normally distributed. Statistical significance was assessed by unpaired Student’s t test (Fig. 2; Fig. 3F; Fig. 4, B and D; Fig. 5, B and G; Fig. 6C), or ANOVA followed by Student-Newman-Keuls post hoc test for multiple comparisons (Fig. 1; Fig. 3B; Fig. 4C; Fig. 5, D and E; Fig. 6A). The null hypothesis of each series of experiments was that the test compound has no influence on the respective parameter. Values of P ≤ 0.05 were considered statistically significant.

Influence of NMDAR Antagonists on Membrane Potential Oscillations. NMDARs are ligand- and voltage-gated ion channels, which allow the permeation of Ca2+ ions. As changes in membrane potential and subsequent Ca2+ influx are the key events for regulation of insulin release, we studied the influence of the NMDAR antagonists on electrical activity. Whole pancreatic islets were cultured for 3 to 4 days on microelectrode arrays to achieve a close contact between islet and electrode. The FOPP, i.e., the period islets display bursts of action potentials, was determined at a glucose concentration of 8 mM, which is close to the threshold concentration for activation of mouse islets. At this glucose concentration, the FOPP varied around 10%-20%, and stimulatory effects were easy to detect. The oscillatory pattern induced by 8 mM glucose was changed to permanent depolarization after application of 100 μM DXM (representative experiment shown in Fig. 2A, lower trace). Corresponding to the results observed for insulin secretion, the low concentration of 10 μM DXM elevated the FOPP significantly less than 100 μM DXM (Fig. 2A, upper trace, Fig. 2B, evaluation of all recordings). Similar results were obtained for DXO (Fig. 2, C and D).

Effects of DXM and DXO on [Ca2+]i in Islets and Single β-Cells. So far, our data support the hypothesis concluded from the experiments with MK-801 (Marquard et al., 2015) that blocking NMDAR augments the glucose-induced depolarization of β-cells. As this is expected to result in a rise in Ca2+ influx, we examined whether changes in electrical activity and glucose-induced insulin secretion are correlated to changes in [Ca2+]i. Treatment of whole islets with DXM (100 μM) abolished the typical oscillatory pattern of [Ca2+]i in the presence of 8 mM glucose and elevated [Ca2+]i compared with controls (Fig. 3A lower vs. upper part, evaluation of all experiments: Fig. 3B). In these experiments, DXM was added to the bath solution 1 hour before and during the initial part of the recording. Of note, [Ca2+]i further increased after washout of DXM, and sometimes fast oscillations reoccurred. Furthermore, analysis of basal [Ca2+]i, at the end of each experiment, in which glucose concentration was lowered from 8 to 0.5 mM, revealed that [Ca2+]i decreased as expected but remained on a higher level in those islets exposed to DXM before compared with controls (Fig. 3B). To test whether DXM elevates [Ca2+]i at a substimulatory glucose concentration, it was added in the presence of 3 mM glucose for 1 hour. Thereafter, the recording was started, and [Ca2+]i was monitored for 15 minutes with DXM still present (see Fig. 3C, lower trace). This maneuver was followed by the washout of DXM and finally by switching the glucose concentration from 3 to 8 mM to confirm the metabolic integrity of the islets. Evaluation of these data showed that DXM increased the basal [Ca2+]i (Fig. 3D). Another series of experiments revealed that basal [Ca2+]i increased in response to acute treatment with DXM (15 minutes), but the effect was much less pronounced (3 mM glucose: F340/380: 0.48 ± 0.10 a.u., +100 μM DXM: 0.50 ± 0.11 a.u., n = 8, P = 0.04, data not shown).

Next, we investigated the acute influence of DXM on [Ca2+]i in glucose-stimulated islets. Remarkably, DXM (100 μM,
10 minutes) exerted a dual effect on the Ca\(^{2+}\) oscillations triggered by 8 mM glucose. Initially, the time \([Ca^{2+}]_c\) remained at the low “interburst” level was prolonged. This was observed consistently: when DXM was added at the beginning, on top of, or at the end of an oscillation. Thereafter, \([Ca^{2+}]_c\) was raised to a plateau with or without small, superimposed oscillations, whereas regular, large oscillations did not reoccur during the application of DXM (Fig. 3E, upper trace). For analysis of the initial effect of DXM, the interburst interval of the glucose-induced oscillations was calculated and compared with the time \([Ca^{2+}]_c\) dropped to the interburst niveau in response to DXM. This period was considerably elevated (control 1.1 ± 0.5 minute vs. DXM 3.4 ± 0.9 minutes, \(n = 14, P = 0.000001\)). Evaluation of the last 5 minutes of drug application showed that, similar to the results obtained with preincubated islets, mean \([Ca^{2+}]_c\) was increased in DXM-treated cells versus control (Fig. 3F, left part).

Analogous experiments were performed with DXO. Acute application of 100 \(\mu\)M DXO to islets stimulated by 8 mM glucose slightly elevated mean \([Ca^{2+}]_c\) (Fig. 3E, lower trace, and Fig. 3F, right part), but the effect was substantially lower compared with DXM (last 5 minutes of application of DXO: 1.1 ± 0.2 a.u., \(n = 14, P = 0.00003\)). In contrast to DXM, DXO did not prolong the first interburst phase after drug application.

**Opposing Effects of DXM and DXO on Membrane Potential in Single β-Cells.** The transient inhibition of Ca\(^{2+}\) oscillations in response to acute application of DXM was

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**Fig. 1.** Effects of DXM and DXO on insulin secretion of mouse islets. (A) Acute treatment (1 hour) with DXM (100 \(\mu\)M) elevates insulin release in the presence of 3, 8, 10, and 15 mM glucose. (B) DXO (100 \(\mu\)M) induces stimulatory effects when exposure time was prolonged to 2.5 hours (1.5 hours preincubation and 1 hour acute application). (C) Insulin secretion (15 mM glucose) is not enhanced by 10 \(\mu\)M DXM but is reduced by 500 \(\mu\)M DXM. (D) Islets stimulated with 15 mM glucose and treated with 10 \(\mu\)M DXO do not show enhanced insulin secretion. (E) DXO and DXM elevate insulin release in the presence of 3 mM glucose and during the first and second phase in response to stimulation with 15 mM glucose (evaluated areas marked in gray). The number of independent islet preparations from different mice is given below each bar. The diagrams in (A–E) summarize the results of all series. Islets were isolated from female/male mice as follows: 10/8 (A), 6/6 (B), 9/9 (C), 5/7 (D), 5/3 (E). *\(P \leq 0.05\); **\(P \leq 0.01\); ***\(P \leq 0.001\) as indicated; \#\(P \leq 0.05\) vs. 10 mM glucose (A) and vs. 500 \(\mu\)M DXM (C); ****\(P \leq 0.001\) vs. 15 mM glucose and all conditions with DXM (A and C), DXO (B and D); \(P \leq 0.05\) (A) and \(P \leq 0.001\) (B) vs. 15 mM glucose. Please note that, for clearness of presentation, only the most relevant significances are included in parts (A, B, and E) of this figure.
unexpected, as inhibition of NMDAR should dampen the negative feedback of Ca^{2+}-dependent activation of repolarizing currents. Therefore, the influence of DXM on Ca^{2+} action potentials and ion channels was investigated in patch-clamp experiments. In the perforated-patch configuration in which cell metabolism is intact, application of DXM (100 μM) to β-cells stimulated by 8 mM glucose led to an initial reduction in the frequency of action potentials (Fig. 4, A and B). β-Cells were hyperpolarized in one out of six experiments, but overall, V_m was unaffected (control: −35 ± 7 mV vs. DXM: −31 ± 6 mV, n = 6, P = 0.11). The inhibiting effect of 100 μM DXM was also present at a glucose concentration of 15 mM. The lower concentration of 10 μM DXM did not affect action potential frequency (Fig. 4C). In agreement with a drop in [Ca^{2+}]_c, DXM (100 μM) partially inhibited the Ca^{2+} current (Fig. 5, A and B). Besides a reduction in peak current (Fig. 5B), the decay of the current tended to be faster after application of DXM. This might indicate an influence on channel inactivation (decay time to 63% of the peak current, control: 40 ± 24 milliseconds, n = 4, + DXM: 29 ± 13 milliseconds, n = 5, P = 0.39).

As this result could not explain why the Ca^{2+}-lowering effect was only transient, it was investigated whether DXM also interacts with K_ATP channels. It was described that NMDA receptor activity contributes to K_ATP channel trafficking and activity, but up to now, there is no information about any direct effects of NMDAR blockers on this ion channel (Shen and Johnson, 2010; Wu et al., 2017). Both compounds, DXM and DXO, inhibited the K_ATP current (Fig. 5, C–F). DXM reduced the K_ATP current dose-dependently (Fig. 5, C and D). The effect was statistically significant at 50 (control: 20 ± 5 pA/pF vs.
DXM 50 μM: 16 ± 5 pA/pF, n = 6, P = 0.0007) and 100 μM (control: 24 ± 5 pA/pF vs. DXM 100 μM: 7 ± 4 pA/pF, n = 5, P = 0.001). After withdrawal of DXM (100 μM), there was a partial recovery in two out of the five experiments, and on average, \( I_{\text{K_{ATP}}} \) current rose to 12 ± 4 pA/pF (n = 5, P = 0.23 vs. DXM). The inhibitory effect of DXM was not antagonized by 100 μM diazoxide (Fig. 5E) (Dabrowski et al., 2003). DXO (100 μM) also lowered the \( I_{\text{K_{ATP}}} \) current to more than 50% of control (Fig. 5, F and G). This effect was not reversible (8 ± 5 pA/pF, n = 5, P = 0.36 vs. DXO). Currents were identified as \( I_{\text{K_{ATP}}} \) currents by application of tolbutamide (100 μM) (Trube et al., 1986) in each recording. To elucidate whether direct inhibition of \( I_{\text{K_{ATP}}} \) channels is a side effect shared by other NMDAR blockers, memantine was tested. \( I_{\text{K_{ATP}}} \) current was reduced to approximately 30% by 100 μM of this drug (Supplemental Fig. 1).

**Contribution of \( I_{\text{K_{ATP}}} \) Channels to the Rise in Insulin Secretion by DXM and DXO.** The data described above show that DXM and DXO directly influence \( I_{\text{K_{ATP}}} \) and Ca\(^{2+}\) channels in addition to their well known function as NMDAR
antagonists. To investigate the significance of inhibition of KATP and/or L-type Ca²⁺ channels for modulation of insulin release, we used islets of SUR1-KO mice that do not express functional KATP channels in their plasma membrane (Seghers et al., 2000). Insulin secretion of SUR1-KO islets stimulated by 15 mM glucose was not elevated by 100 μM DXM (Fig. 6A).

[Ca²⁺]c measurements with SUR1-KO islets even showed a substantial decrease in mean [Ca²⁺]c when DXM was added to the bath solution (Fig. 6B, upper graph, and Fig. 6C, left). This loss of stimulatory effects in SUR1-KO islets demonstrates that inhibition of KATP channels is the underlying mechanism for the elevation of insulin release by DXM. In comparison, DXO clearly tended to raise insulin secretion in SUR1-KO islets when stimulated with 15 mM glucose (Fig. 6A, grey bar). In addition, determination of the acute effect of DXO on [Ca²⁺]c in SUR1-KO islets even showed a slight but statistically significant increase by 100 μM DXO (Fig. 6B, lower graph, and Fig. 6C, right). These data suggest that additional KATP channel–independent pathways contribute to the insulinotrophic effect of DXO.

Discussion

DXM and DXO can increase glucose-induced insulin secretion and were proposed to be potential drugs for treatment of type 2 diabetes mellitus. How NMDAR might modulate β-cell function was investigated with MK-801 (Marquard et al., 2015). Controversial results exist regarding the effect of this compound on insulin release. Although one group (Marquard et al., 2015) detected an elevation of glucose-stimulated insulin secretion, others describe no effect during acute application of MK-801 (Bertrand et al., 1992; Chan et al., 1997; Patterson et al., 2016) or even inhibition after prolonged exposure (Patterson et al., 2016). Consequently, it is difficult to conclude from experiments with MK-801 the mode of action of DXM and DXO. Our results reveal that DXM and its metabolite DXO elevate electrical activity, [Ca²⁺]c, and insulin secretion. The most efficient concentration in our experiments was 100 μM. This concentration will certainly not be reached in the plasma, but cumulative effects might occur in tissues during long-term use. DXO (10 μM) led to inconclusive results in our study: A rise in secretion was observed in two out of the six mouse preparations, whereas the others showed no or only a negligible response. This result contrasts with Marquard et al. (2015) and cannot be explained by differences in methodology. Of note, Suwandhi et al. (2018) describe a clear stimulatory trend for 10 μM DXO but also no significant effect.

At first glance, our data support the hypothesis that NMDAR antagonists enhance insulin release via the triggering pathway regulated by the plasma membrane potential. Mechanistically, the NMDAR can interact with insulin secretion by several pathways. Wu et al. reported increased trafficking of K_ATP and Kᵥ2.1 channels to the membrane surface in response to NMDA (Wu et al., 2017). The rise in Ca²⁺ influx via NMDAR could also depolarize mitochondria, thereby increasing the open probability of K_ATP channels, as described for Ca²⁺ influx via voltage-regulated Ca²⁺ channels (Caᵥ channels) (Drews et al., 2015). Finally, increased local [Ca²⁺]c could activate Ca²⁺-regulated K⁺ channels (Kᵥ channels). Together with the hyperpolarizing current through K_ATP channels, opening of Kᵥ channels is known to initiate the end of each burst phase during the typical oscillations of Ca²⁺ action potentials in glucose-stimulated β-cells.

Fig. 4. In contrast to DXO, DXM reduces Ca²⁺ action potentials in glucose-stimulated β-cells. (A) Representative membrane potential recording of a pancreatic β-cell in the perforated-patch configuration. DXM (100 μM) decreases the frequency of action potentials (8 mM glucose). (B) Summary of all experiments represented in (A). (C) Summary of analogous experiments with 15 mM glucose. A lower concentration of DXM (10 μM) does not affect the number of Ca²⁺ action potentials (APs). (D) Similar experiments as in (A) but with DXO. DXO (100 μM) has no effect on Ca²⁺ action potentials. The number in brackets represents the number of experiments with different cells from three mice. Islet cells were isolated from female/male mice as follows: 2/1 (B), 4/3 (C), 2/1 (D). *P < 0.05; **P < 0.01.
glucose-stimulated pancreatic islets (Düfer et al., 2009; Rorsman and Ashcroft, 2018). Consequently, inhibition of NMDARs should impede repolarization of the β-cell and prolong the burst phases of electrical activity. For MK-801, it was suggested that the stimulatory effect requires KATP channels (Marquard et al., 2015). To elucidate whether this also applies to DXM and DXO, islets of SUR1-KO mice were used. The two NMDAR antagonists acted differently in KATP channel–deficient islets. Concomitant with the loss of any positive influence on [Ca^{2+}]_{i}, insulin release was not stimulated by DXM in SUR1-KO islets. By contrast, DXO still elevated [Ca^{2+}]_{i}, although the effect was less pronounced compared with wild-type islets. The effect of DXO on insulin release was more variable in SUR1-KO islets. Stimulation was observed in 7 out of 10 experiments. Statistical significance was reached when tested separately versus control by a Student’s t test but not in the ANOVA group comparison.

So far, it is obvious that K_{ATP} channels play a central role for the efficacy of DXM and—together with other mechanisms—are involved in the mode of action of DXO. To clarify whether NMDAR is the predominant target for modulation of K_{ATP} channel activity, we investigated the direct influence on other ion channels. Our experiments show for the first time that DXM and DXO both directly inhibit KATP channels. The effect of DXM was resistant to diazoxide. This differs from the characteristics of established KATP channel blockers (Rorsman and Trube, 1986) but was reported, e.g., for the experimental compound phentolamine (Plant and Henquin, 1990). As the KATP channel blockade was very fast and strong, we assume that this direct interaction is much more important for β-cell stimulation than indirect pathways induced by inhibition of NMDAR. Direct inhibition of K^+ currents by DXM was reported for K_{1,3} channels (Lee et al., 2011). These channels

Fig. 5. DXM acutely decreases Ca^{2+} peak currents, and both compounds inhibit K_{ATP} channels of murine β-cells. (A) Representative voltage-clamp recording in the whole-cell configuration shows a decrease of the maximal Ca^{2+} current during administration of DXM (solid, black line) compared with the control condition (solid, gray line). (B) Summary of all experiments represented in (A). DXM (100 μM) reduces the Ca^{2+} current about 25%. (C and F) Representative recordings of K_{ATP} current measured in the whole-cell configuration. Administration of DXM (C) or DXO (F) directly decreases the K_{ATP} current. The current was identified as K_{ATP} current by the specific K_{ATP} channel inhibitor tolbutamide (Tolb; 100 μM). (D) Dose-response curve for the direct effect of DXM (10, 20, 50, and 100 μM) on K_{ATP} current. Statistically significant inhibition occurred with 50 and 100 μM. (E) Lack of antagonism of the DXM-induced inhibition by diazoxide (100 μM). (G) Summary of all experiments with 100 μM DXO. The number in brackets indicates the number of experiments with different cells from three mice. Islet cells were isolated from female/male mice as follows: 4/1 (B), 7/3 (D), 0/3 (E), 3/0 (G). *P ≤ 0.05; **P ≤ 0.01; ***P ≤ 0.001 vs. all other conditions, *P ≤ 0.05 vs. control and 20 μM DXM, ##P ≤ 0.01 vs. control.
seem to be irrelevant for glucose-stimulated insulin secretion but are associated with regulation of insulin sensitivity (Choi and Hahn, 2010). Taken together, DXM may improve glycemic control by a dual mechanism: an increase in insulin sensitivity and a rise in insulin secretion. In principle, such a combination is not bad, but direct blocking of KATP channels in \( \beta \)-cells always bears the risk of hypoglycemia. This apprehension is supported by results of Suwandhi et al. (2018), showing a statistically significant membrane depolarization of \( \beta \)-cells with DXO at 2 mM glucose, and by our observation that DXO and DXM extremely elevate insulin release in the presence of 3 mM glucose. DXO stimulates insulin release at this low glucose concentration but is most effective in the presence of 8 and 10 mM glucose. This is in line with a compound inhibiting K\(_{\text{ATP}}\) channels. At 15 mM glucose, when the majority of K\(_{\text{ATP}}\) channels is already closed, the effect of channel blockers (sulfonylureas or glinides) is less pronounced (Joost and Hasselblatt, 1979; Ikenoue et al., 1997), similar to our results with DXO.

Apart from the problem of nutrient-independent insulin release, limiting the therapeutic potential of DXM and DXO equally, our study revealed a Ca\(^{2+}\)-lowering effect of DXM. The drop in [Ca\(^{2+}\)]\(_{\text{c}}\) was relatively short and transient in wild-type islets but persisted during the whole application period in sulfonylurea receptor 1–deficient islets. As this is compatible with neither direct inhibition of K\(_{\text{ATP}}\) channels nor interruption of the indirect negative feedback via NMDAR and K\(_{\text{ATP}}\) or KCa channels, DXM must address an additional target. Indeed, we could show that DXM reduces the peak current through L-type Ca\(^{2+}\) channels. Inhibition of Cav channels by DXM (and DXO) was described for neuronal cells (Carpenter et al., 1988; Netzer et al., 1993). Our patch-clamp recordings, in which intracellular Ca\(^{2+}\) was clamped to a low level, suggest an accelerated inactivation by this compound, but further studies are necessary to address this point in detail. Affinity assays showed a 10-fold-higher affinity to Ca\(_{\text{v}}\) channels for DXM versus DXO. Furthermore, the affinity of DXM to Ca\(_{\text{v}}\) channels is 1.7-fold higher than to NMDARs (Jaffe et al., 1989). In agreement with these differences in the inhibitory potencies, DXM lowered the frequency of Ca\(^{2+}\) action potentials in patch-clamped \( \beta \)-cells, whereas DXO neither affected action potentials nor reduced [Ca\(^{2+}\)]\(_{\text{c}}\). In SUR1-KO islets, DXM lowered [Ca\(^{2+}\)]\(_{\text{c}}\), but the remaining level was still above basal, and glucose-stimulated insulin release was not
negatively affected. Of note, the high concentration of 500 μM DXM dramatically decreased glucose-stimulated insulin release of wild-type islets. This suggests that the inhibitory effect on L-type Ca^{2+} channels outweighs all other signaling pathways that DXM acts on when the drug accumulates in pancreatic tissue or is severely overdosed.

DXM and DXO also differ in their ability to bind to NMDAR: the affinity of DXO to the NMDAR was reported to be 6.5 times higher compared with DXM (Jaffe et al., 1989), and in contrast to DXM, DXO still raised [Ca^{2+}]_i, in SUR1-KO islets. This provides evidence for the potential of NMDAR antagonists to improve β-cell function apart from any (direct or indirect) interaction with K_{ATP} channels. Reduced activation of KCa channels might play an important role in this scenario. NMDA receptors couple to or are part of a Ca^{2+}-mediated feedback loop with KCa channels in neurons (Isaacson and Murphy, 2001; Ngo-Anh et al., 2005). One candidate might be the SK4 channel (synonymous, KCa3.1 channel), which is known to be part of the slowly rising K^+ currents terminating burst phases in oscillating β-cells. Eliminating this component of the so-called K_{slow} current dampens the negative-feedback mechanisms maintaining oscillations and elevates [Ca^{2+}]_i, thereby fostering insulin release (Düfer et al., 2009). Targeting this pathway has the big advantage that it is not operative under basal [Ca^{2+}]_i, i.e., without nutrient stimulation. In this context, it is astonishing that blocking NMDAR by MK-801 was completely ineffective in Kir6.2 knockout islets (Marquard et al., 2015). Possibly, loss of K_{ATP} channels affects the alternative pathways recruited to preserve glucose-regulated hormone secretion differently in the two mouse models. This assumption is supported by the observation that insulin release of islets of Kir6.2 knockout mice is less sensitive to glucose (Miki et al., 1998; Marquard et al., 2015) compared with islets of SUR1-KO mice (Fig. 6 and (Haspel et al., 2005)). Furthermore, it was shown for β-cells of SUR1-KO mice that KCa channels could be activated by forced Ca^{2+} influx (Haspel et al., 2005), but it is not known whether this also applies to Kir6.2-deficient β-cells.

DXM was already tested in combination with sitagliptin in a small clinical trial. DXM (independent of dose) numerically reduced maximum blood glucose concentration in response to an oral glucose tolerance test (Marquard et al., 2016). Although the effect was not statistically significant, the results may encourage people to use DXM as an oral antidiabetic drug. Strikingly, development of insulin-dependent diabetes mellitus was reported in a pilot study investigating the therapeutic potential of DXM for treatment of children with severe meningitis (Konrad et al., 2000). With respect to the risk of negative side effects, one can argue that DXO, as the main active metabolite, would be the leading compound reaching the pancreatic tissue or is severely overdosed.

In summary, our data indicate that the NMDAR influences glucose-mediated stimulus-secretion coupling by K_{ATP} channels, depending on both positive and negative feedbacks. With respect to DXM and DXO, the stimulatory mode of action is based on direct inhibition of K_{ATP} channels rather than on NMDAR blockade. To investigate the role of NMDAR in β-cell (patho)physiology and to further elucidate the therapeutic potential of NMDAR inhibitors, novel compounds that, e.g., specifically target NMDAR subunits or are unrelated to the morphinan core structure have to be developed.

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Authorship Contributions

Participated in research design: Gresch, Düfer.

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References


Chan SLF, Fallett AL, Clevs J, Ramsden CA, and Morgan NG (1997) Evidence that the ability of imidazoline compounds to stimulate insulin secretion is not due to interaction with α-receptors. Eur J Pharmacol 323:241–244.


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**Supplemental Figure 1**

Memantine (100 µM) acutely decreases $K_{\text{ATP}}$ currents of murine β-cells. A) Representative recording of $K_{\text{ATP}}$ current measured in the whole-cell configuration. Administration of 1 µM memantine had no effect while 100 µM decreased the $K_{\text{ATP}}$ current. The current was identified as $K_{\text{ATP}}$ current by the specific $K_{\text{ATP}}$ channel inhibitor tolbutamide (100 µM). B) Summary of all experiments. Different concentrations were not always applied in the same recording. The number in brackets indicates the number of experiments with different cells from 3 mice. Islets were isolated from female/male mice as follows: 2/1 (B). **p≤0.01.