学 位 論 文

Acetaldehyde administration induces salsolinol formation in vivo in the dorsal striatum of Aldh2-knockout and C57BL/6N mice

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Acetaldehyde administration induces salsolinol formation in vivo in the dorsal striatum of Aldh2-knockout and C57BL/6N mice

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ABSTRACT

Acetaldehyde (AcH) and salsolinol play important roles in the central effects of ethanol. This study aimed to investigate the effect of administration of AcH on dopamine (DA), DA-derived salsolinol and serotonin (5-HT) levels in the dorsal striatum of Aldh2-knockout (Aldh2-KO) and C57BL/6 N (WT) mice. Animals were treated with AcH (50, 100 and 200 mg/kg) intraperitoneally and dialysate levels of DA, 5-HT and salsolinol were determined using in vivo microdialysis coupled with HPLC-ECD. Salsolinol was first detected at 20 min after AcH administration, and reached its peak concentration (WT mice: 0.29 ± 0.22 pg/μl; Aldh2-KO mice: 0.63 ± 0.17 pg/μl) at 25 min in the 200 mg/kg AcH group, before decreasing rapidly and reaching zero at approximately 55–80 min. Treatment with 100 and 200 mg/kg AcH increased levels of salsolinol in both WT and Aldh2-KO mice, with 200 mg/kg AcH inducing a higher level of salsolinol in Aldh2-KO mice than in WT mice. Treatment with 50 mg/kg AcH produced a small increase in salsolinol levels in Aldh2-KO mice, whereas no elevation of salsolinol was detected in WT mice. The increase in salsolinol formation was found to occur a dose-dependent manner in both genotypes. Administration of AcH and the subsequent changes in salsolinol concentrations did not change DA or 5-HT levels in either genotype. Our study suggests that AcH dose-dependently increases the formation of salsolinol in the dorsal striatum of mice, which provides further support for the role of AcH in salsolinol formation in the animal brain.

1. Introduction

Acetaldehyde (AcH) is a highly toxic metabolite of ethanol (EtOH) that has several neurochemical, behavioral and neurotoxic effects. Aldehyde dehydrogenase 2 (ALDH2) plays an important role in detoxifying AcH, but approximately 40% of the East Asian population are deficient in the ALDH2 enzyme [1,2]. This deficiency may lead to AcH accumulation in the blood and brain after drinking EtOH. In the periphery, AcH has a primarily aversive effect [3], whereas in the brain, it exerts reinforcing effects [4,5]. Moreover, AcH is involved in mediating the sedative, amnestic and stimulant properties of EtOH [6]. Following EtOH consumption, AcH interacts with endogenous neurotransmitters such as dopamine (DA) and serotonin (5-HT) to form tetrahydroisoquinoline alkaloids and tetrahydro-β-carboline [7–9].

Salsolinol (1-methyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline) is a psychoactive compound formed through the non-enzymatic condensation of AcH with DA in the peripheral and central nervous system of mammals, including humans [10–12]. Both AcH and salsolinol are suggested to be involved in mediating a number of EtOH-induced pharmacological effects, especially the reinforcing effects of EtOH [13–15]. Salsolinol has been proposed to play a role in the etiology of alcoholism [7] and the rewarding properties of EtOH [16]. Prior studies in animals have revealed that salsolinol promotes alcohol consumption [16,17] and that microinjection of salsolinol into the posterior ventral tegmental area (VTA) of rats induces a conditioned place preference [18]. However, salsolinol has also been found to exert neurotoxic

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effects which alter the normal function and survival of DA neurons [19,20] and has been proposed to be an endogenous compound contributing to Parkinson's disease [21,22].

Research has shown that AcH and salsolinol are formed following EtOH consumption, with salsolinol generated from DA and AcH. Both AcH and salsolinol exhibit potent reinforcing properties within the mesolimbic DA reward pathway [23]. Despite extensive research into the effects of AcH and salsolinol, no study to date has investigated the effect of AcH on the formation of salsolinol in the dorsal striatum of Aldh2-knockout (Aldh2-KO) mice, a model of ALDH2 deficiency in humans. To investigate this, we administered saline (control) or AcH (50, 100, and 200 mg/kg) intraperitoneally (i.p.) to C57BL/6 N (WT) and Aldh2-KO mice. Concentrations of DA, 5-HT and salsolinol were measured using in vivo microdialysis in combination with high-performance liquid chromatography and electrochemical detection (HPLC-ECD) in awake, freely moving animals.

2. Materials and methods

2.1. Animals

All animal experiments were approved by the Kagawa University Animal Investigation Committee. Aldh2-KO mice were generated as previously reported [24]. These mice were maintained and backcrossed with the C57BL/6 N strain for more than 10 generations. They have the same genetic background, except for Aldh2. Two eight-week-old male/female pairs were obtained from the Department of Social Medicine of Saga University in Japan and were bred at the Kagawa University animal facility. Breeding pairs from this strain were used to generate the experimental groups. WT mice have the same genetic background as C57BL/6 N mice. These mice were purchased from CLEA Japan (Tokyo, Japan). All experiments were conducted with male mice. Each was 10–12 weeks in age and weighed 24–28 g. All animals were housed in a regulated temperature (21 ± 3 °C), humidity (50–70%) and light (12-h light-dark cycle) conditions. Aldh2-KO and WT mice were each divided into four experimental groups: (a) Saline (control, n = 5), (b) AcH (50 mg, n = 5), (c) AcH (100 mg/kg, n = 5) (d) AcH (200 mg/kg, n = 5). A stock solution of AcH (4%, w/v) were prepared in 0.9% saline, and all injections were administered i.p. in a volume of 10 ml/kg. AcH was purchased from Merck-Schuchardt, Hohenbrunn, Germany and used was of the highest quality available.

2.2. Surgical procedure

Each mouse was anesthetized with an i.p. administration of a mixture of medetomidine hydrochloride (0.3 mg/kg), midazolam (4 mg/kg) and butorphanol tartrate (5 mg/kg) [25]. Animals were placed in a stereotaxic apparatus (SR-5 N, Narishige Scientific Instrument Lab, Tokyo, Japan) for the surgical implantation of a guide cannula (AG-4, Eicom, Kyoto, Japan). The guide cannula was positioned above the right dorsal striatum using the following coordinates: anterior, 0.2 mm to the bregma; lateral, 1.8 mm; depth, 2 mm according to the atlas of Paxinos and Franklin [26]. The guide cannula was secured to the skull with dental cement anchored by a stainless-steel screw, and then a dummy cannula was inserted into the guide cannula. After surgery, mice were kept in a separate cage where food and water were available ad libitum. The mice were kept warm until fully ambulatory using a heat lamp. The mice were allowed to recover for 24 h after stereotaxic surgery.

2.3. Microdialysis

In the morning on the day of the analysis, the dialysis experiments commenced with the insertion of a probe (A-I-4-2, Eicom) equipped with an active dialysis membrane (2 mm long; inner diameter, 0.20 mm; outer diameter, 0.22 mm; cutoff value, 50 kDa) constructed from hemicellulose dialysis tubing. Mice were caged individually in acrylic cages (width 30 cm, length 35 cm, depth 30 cm). The probe's inlet was perfused continuously with Ringer's solution (147 mM NaCl, 4 mM KCl, 2.25 mM CaCl2, pH 6.4) at a constant rate of 1 μl/min using a 1.0-ml gas-tight syringe (Hamilton, Reno, NV, USA). Samples were collected at 5 min intervals throughout the experiment. Immediately after obtaining a stable 4-sample baseline of DA and 5-HT, mice were injected saline as a control and AcH (50, 100 or 200 mg/kg). The basal level (100%) was defined as the average output of four consecutive samples that did not differ by more than 4%. The in vitro recovery of DA and 5-HT from 2 mm membrane length probes was 15% [27] and the dialysate levels of DA and 5-HT were not corrected for the recovery. The in vitro recovery of salsolinol (13.5%) was measured using three microdialysis probes (A-I-4-2, Eicom) but data were not corrected for the recovery.

2.4. HPLC conditions

In order to determine the concentrations of DA and 5-HT in the dialysate samples, we used an HPLC system equipped with an ECD-300 (Eicom) and an autosampler (EAS-20, Eicom). The main operative conditions for HPLC were as follows: column (Eicom- PP-ODS II; 4.6 mm × 30 mm), oven temperature of 25 °C, detector, oxidation potential (+ 400 mV versus Ag/AgCl reference analytical electrode), mobile phase: 2% MeOH/100 mM phosphate buffer (pH 5.4) containing 50 mg/l disodium EDTA and 500 mg/l sodium decane-1-sulfonate at a flow rate of 0.5 ml/min. The dialysis samples were collected in 5 min intervals by an autoinjector connected to the automated HPLC-ECD system. The chromatograms were recorded with a PowerChrom (AD Instruments, Sydney, Australia).

2.5. Statistical analysis

The results are expressed as the mean ± SD. DA and 5-HT levels were analyzed by using a two-way analysis of variance (ANOVA) with repeated measures over time with treatments (control, AcH) as the independent factors. Salsolinol levels were analyzed by one-way ANOVA over time with treatments (control, AcH) as the independent factors. Student's *t*-test was used for comparisons of salsolinol levels between two genotypes. A post hoc Tukey-Kramer test was used for multiple comparisons. The level of significance was set at $P < 0.05$. All analyses were conducted using the statistical program StatView (J-4.5, Abacus Concepts, Berkeley, CA, USA).

3. Results

Fig. 1 shows the effects of AcH (50, 100, and 200 mg/kg) on extracellular levels of DA, 5-HT and salsolinol in WT mice. Salsolinol was first detected at 20 min after AcH administration, reaching its peak concentration 0.14 ± 0.01 and 0.29 ± 0.22 pg/μl at 25 min in the 100 and 200 mg/kg AcH groups, respectively, before decreasing gradually and reaching zero after approximately 55 min (Fig. 1C). No elevation of salsolinol was detected in the 50 mg/kg AcH group. Analysis by one-way ANOVA revealed that the treatment with 200 mg/kg AcH resulted in a significant increase in salsolinol levels (main effect of treatment at 20 min: df 3,16; $F = 5.339$; $p < 0.05$; 25 min: df 3,16; $F = 8.194$; $p < 0.01$; 30 min: df 3,16; $F = 9.987$; $p < 0.001$; 35 min: df 3,16; $F = 7.325$; $p < 0.01$) compared to the control and 50 mg/kg AcH groups. Two-way ANOVA revealed that treatment with 50, 100 or 200 mg/kg AcH had no significant main effect of treatment on DA (df 3,216; $F = 0.219$; $p = 0.881$) or 5-HT (df 3,216; $F = 0.826$; $p = 0.505$) levels compared with the control group (Fig. 1A, B).

Fig. 2 shows the effects of AcH (50, 100, and 200 mg/kg) on extracellular levels of DA, 5-HT and salsolinol in Aldh2-KO mice. Salsolinol was first detected at 20 min after AcH administration, reached its peak concentration 0.025 ± 0.02, 0.14 ± 0.05, 0.63 ± 0.17 pg/μl at

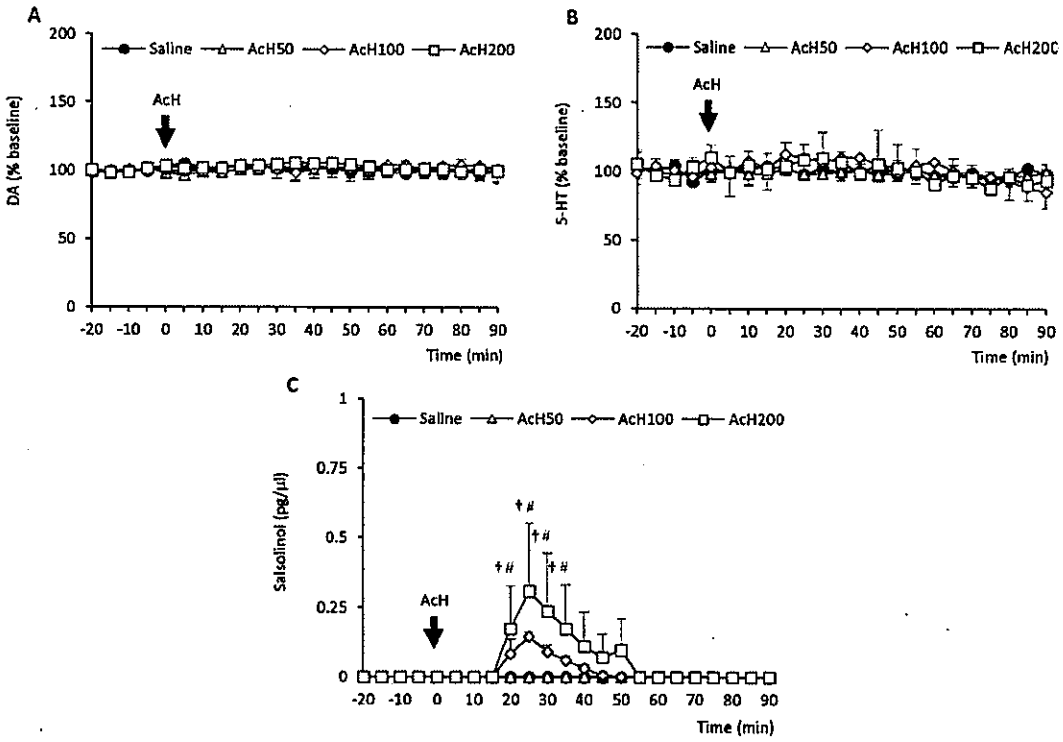

Fig. 1. The effects of 50, 100 and 200 mg/kg AcH on DA (A) and 5-HT (B) levels and time course of changes in salsolinol levels (C) in the striatal dialysates of WT mice (n = 5). The arrow shows the administrations of AcH. #P < 0.05, for difference in salsolinol between the 200 mg/kg AcH and control groups; †P < 0.05, for difference in salsolinol between the 200 and 50 mg/kg AcH groups. DA, dopamine; 5-HT, serotonin; AcH50, AcH 50 mg/kg AcH100, AcH 100 mg/kg; AcH200, AcH 200 mg/kg; AcH; acetaldehyde.

25 min in the 50, 100 and 200 mg/kg AcH groups, respectively, before gradually decreasing and reaching zero after approximately 80 min (Fig. 2C). Analysis by one-way ANOVA revealed that treatment with 200 mg/kg AcH resulted in a significant increase in salsolinol levels (main effect of treatment at 20 min: df 3,16; $F = 29.264$; $p < 0.001$; 25 min: df 3,16; $F = 48.487$; $p < 0.001$; 30 min: df 3,16; $F = 27.587$; $p < 0.001$; 35 min: df 3,16; $F = 28.274$; $p < 0.001$; 40 min: df 3,16; $F = 17.194$; $p < 0.001$; 45 min: df 3,16; $F = 16.610$; $p < 0.001$; 50 min: df 3,16; $F = 9.346$; $p < 0.001$; 55 min: df 3,16; 8.262; $p < 0.01$; 60 min: df 3,16; $F = 6.407$; $p < 0.01$; 65 min: df 3,16; 12.540; $p < 0.001$; 70 min: df 3,16; $F = 23.029$; $p < 0.001$; 75 min: df 3,16; $F = 19.027$; $p < 0.001$) compared to the control, 50 and 100 mg/kg AcH groups. We found that formation of salsolinol after treatment with 200 mg/kg AcH was higher in Aldh2-KO mice ($p < 0.05$) than in WT mice. Two-way ANOVA revealed that treatment with 50, 100 or 200 mg/kg AcH had no main effect of treatment on DA (df 3,216; $F = 1.818$; $p = 0.198$) or 5-HT (df 3,216; $F = 3.776$; $p = 0.041$) levels compared with the control group, but there was a significant interaction between treatment × time (df 3,54; $F = 2.324$; $P < 0.001$) (Fig. 2A, B).

4. Discussion

The purpose of this study was to explore the effect of systemic administration of AcH in mice on the concentration of DA and 5-HT and the formation of DA-derived salsolinol in the dorsal striatum, the brain area known to contain the highest levels of DA. To investigate this, we administered three different doses of AcH (50, 100, and 200 mg/kg) in Aldh2-KO and WT mice. Salsolinol was first detected at 20 min following administration of AcH, reached its peak concentration at 25 min, before gradually decreasing and reaching zero at around 55–80 min. Formation of salsolinol in the dorsal striatum of both Aldh2-KO and WT mice was dose-dependently increased by AcH, with the increase in salsolinol formation greater in Aldh2-KO mice than WT mice in the 200 mg/kg AcH group. None of the doses of AcH administered nor the subsequent changes in salsolinol concentrations altered levels of DA or 5-HT in either genotype. These in vivo results are the first to measure salsolinol formation in the dorsal striatum of Aldh2-KO mice following direct administration of AcH.

Previously, we measured brain AcH concentrations in mice following direct administration of AcH [28]. These earlier results showed that AcH levels are more than two-fold higher in the brains of Aldh2-KO mice compared to those in WT mice. This elevated levels of AcH in Aldh2-KO mice were probably due to ALDH2, which is the enzyme most likely to responsible for the majority of AcH oxidation. Although AcH concentration was not measured in the current study, we expect that the data would be similar to those we reported in our previous study. AcH levels used in this experiment were much greater than those achieved naturally, and such high levels of AcH in the blood may therefore enter the brain.

Several prior studies from our group have demonstrated salsolinol formation in the rat brain. In these studies, AcH was either directly perfused to a localized site in the brain (250, 500 and 1000 μM) or the potent ALDH inhibitor cyanamide was administered (50 mg/kg) followed by EtOH (1 g/kg) [29,30]. In the current study, we administered AcH (50, 100, and 200 mg/kg) i.p. to Aldh2-KO and WT mice. We used Aldh2-KO mice as a model of humans lacking ALDH2, which results in high AcH accumulation after EtOH intake [31]. We show that 50, 100, and 200 mg/kg AcH increased salsolinol formation in the dorsal striatum of Aldh2-KO mice, whereas only 100 and 200 mg/kg AcH were sufficient to increase salsolinol formation in WT mice (Figs. 1 and 2C). The greater increase of salsolinol levels in the 200 mg/kg AcH group in Aldh2-KO mice was probably due to the higher concentration of AcH in the brain. There was no significant difference in salsolinol formation in

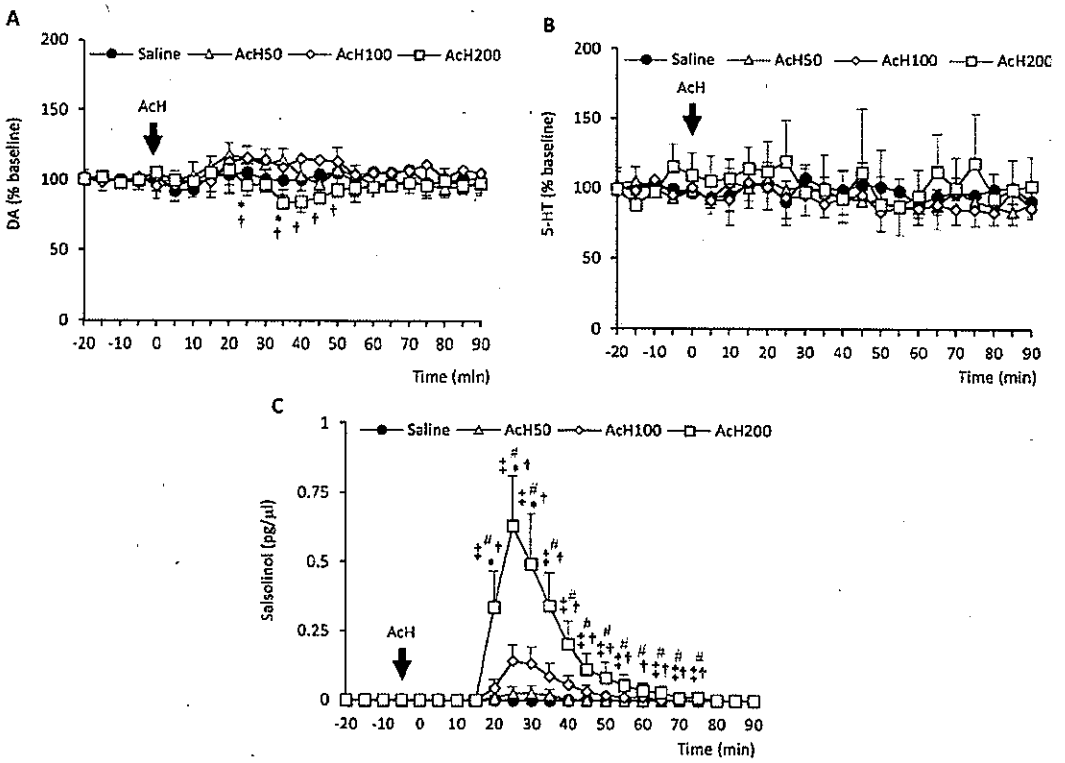

Fig. 2. The effects of 50, 100 and 200 mg/kg AcH on DA (A) and 5-HT (B) levels and time course of changes in salsolinol levels (C) in the striatal dialysates of Aldh2-KO mice (n = 5). The arrow shows the administrations of AcH. *P < 0.05, for difference in DA between the 200 and 50 mg/kg AcH groups; †P < 0.05, for difference in DA between the 200 and 100 mg/kg AcH groups. #P < 0.05, for difference in salsolinol between the 200 mg/kg AcH and control groups; †P < 0.05, for difference in salsolinol between the 200 and 50 mg/kg AcH groups; ‡P < 0.05, for difference in salsolinol between the 200 and 100 mg/kg AcH groups; *P < 0.05, for difference in salsolinol between WT and Aldh2-KO mice. DA, dopamine; 5-HT, serotonin; AcH50, AcH 50 mg/kg AcH100, AcH 100 mg/kg; AcH200, AcH 200 mg/kg; AcH; acetaldehyde.

the 100 mg/kg AcH group between Aldh2-KO and WT mice, although brain AcH levels differ significantly between the two genotypes in this group as we observed previously [28]. The reason for this discrepancy is unknown. Indeed, 50 mg/kg AcH did not significantly increase salsolinol concentration in WT mice, likely due to AcH levels remaining low.

The concentration of salsolinol reached a maximum of 0.63 and 0.29 pg/μl in Aldh2-KO and WT mice, respectively, at 25 min in the 200 mg/kg AcH group. Previously, brain AcH levels reached a sharp peak at 5 min and then quickly declined over 60 min [28]. We found a time lag between peak concentrations of AcH and salsolinol in the brain after administration of AcH. This suggests that there is a delay in the formation of salsolinol following the reaction between AcH and DA. We also found that AcH caused a dose-dependent increase in salsolinol levels in both genotypes, in agreement with a previous study which suggests that the magnitude of salsolinol levels in rat striatum depend on the blood concentration of AcH [32]. Therefore, the current findings in combination with past studies provide further support for the hypothesis that AcH is a key mediator in the formation of salsolinol in the animal brain.

The role of AcH in DA and 5-HT metabolism is still a controversial matter of debate. A number of studies have shown that the effects of AcH on extracellular DA and 5-HT are likely depend on factors such as the concentration of AcH and the route of AcH administration. For example, administration of AcH (20 mg/kg) via gastric gavage [33] or via microinjection into the VTA (23 and 90 μM) [23] were found to increase DA levels in the shell of nucleus accumbens (NAc) in freely moving rats, and increase the activity of mesoaccumbens DA neurons. However, the DA levels in the striatum remained almost unchanged following chronic treatment with AcH [34]. A recent study showed that perfusion of 200 or 500 μM AcH to the dorsal striatum via a microdialysis probe decreases both DA and 5-HT levels in Aldh2-KO mice, but not WT mice [35]. Similarly, local perfusion of 1000 μM AcH (but not 500 μM) was found to cause a significant decrease in DA levels in the striatum of rats [30].

The present study in WT mice showed that direct administration of AcH (50–200 mg/kg) has no effect on extracellular levels of DA and 5-HT in the dorsal striatum (Figs. 1 and 2A–B). A significant but inconsistent decrease in DA was found in Aldh2-KO mice in the 200 mg/kg AcH group compared to the 50 and 100 mg/kg AcH groups where no changes in DA and 5-HT levels were detected compared with controls (Figs. 1 and 2A–B). The decrease in DA at the high dose of AcH in comparison to low-medium doses is probably due to the biphasic pattern of the effect of AcH [36]. Our finding of a lack of eff ect of AcH on DA and 5-HT is in agreement with previous work showing no significant effect of AcH administration on extracellular levels of DA or 5-HT in either the striatum or the NAc of rats [29]. In this study, the authors injected cyanamide (50 mg/kg), a potent ALDH inhibitor, plus EtOH (1 g/kg) i.p. to induce AcH formation in the blood and brain. Similarly, another study showed that AcH (1–200 μM) administered locally in the NAc produced no change in accumbal DA levels in rats [37]. There are two possible explanations for the lack of a consistent effect of AcH on DA. First, it is possible that DA is quickly sequestered by available AcH to form salsolinol, thus meaning the increased DA cannot be detected. Second, AcH might disrupt the extracellular environment in such a way that increases in DA might be hindered [37]. Alternately, the applied doses of AcH may only have been sufficient to produce a moderate effect. The differential effects of AcH at low, medium and high doses may be because very low doses of AcH are stimulatory, whereas medium and higher doses are without effect and depressive,

respectively.

In conclusion, AcH increased the formation of salsolinol in the dorsal striatum of both Aldh2-KO and WT mice, but AcH-induced salsolinol levels were higher in Aldh2-KO mice than WT mice. Administration of AcH and the subsequent changes in salsolinol concentration did not alter levels of DA or 5-HT in either genotype. This is the first in vivo report on salsolinol formation in the dorsal striatum of Aldh2-KO mice following direct administration of AcH, and its findings support the hypothesis that AcH plays a key role in salsolinol formation in the animal brain.

Disclosure statement

The authors declare that there is no conflict of interest regarding the publication of this paper.

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