RESEARCH PAPER

Acta Neurobiol Exp 2024, 84: 256–265
DOI: 10.55782/ane-2024-2585

Microdialysis perfusion of COA-Cl enhances dopamine metabolism in the dorsal striatum of freely moving mice

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We performed a microdialysis study to examine the effects of local perfusion of COA-Cl on the extracellular levels of dopamine (DA) and its metabolites in the dorsal striatum of mice *in vivo*. The mice were perfused with Ringer's solution (control) and COA-Cl (0.05, 0.1, or 0.5 mM) into the dorsal striatum. Dialysate samples were collected every 30 min and then analyzed using high-performance liquid chromatography coupled with an electrochemical detector. We found that local perfusion of COA-Cl (0.1 or 0.5 mM) into the dorsal striatum of living mice produced a significant and dose-dependent increase in extracellular levels of DA, 3-methoxytyramine (3-MT), and homovanillic acid (HVA), where only 0.5 mM COA-Cl increased dihydroxyphenylacetic acid (DOPAC) levels. However, 0.05 mM of COA-Cl did not significantly affect either DA levels or its metabolites. Then, we administered the monoamine oxidase (MAO) inhibitor clorgyline alone or in combination with COA-Cl (0.1 mM) to test whether COA-Cl-induced increases in DOPAC and HVA are mediated by increased MAO activity. Clorgyline alone increased 3-MT levels and decreased DOPAC and HVA levels but not DA levels. When combined with COA-Cl, clorgyline increased 3-MT levels and reversed the decrease in DOPAC and HVA levels caused by clorgyline. The increase in DA metabolism induced by COA-Cl suggests that some DA was further metabolized into DOPAC, 3-MT, and HVA. This indicates that COA-Cl plays a role in DA metabolism *via* increased DA release and/or activation of MAO, offering new insights into the effects of COA-Cl on DA metabolism in the brain.

Key words: COA-Cl, dopamine metabolism, reverse microdialysis, striatum

INTRODUCTION

Dopamine (DA) is a major modulatory neurotransmitter in the brain has various functions, including motor control, mood regulation, learning, and reward processing (Bromberg-Martin et al., 2010; Radwan et al., 2019). The highest levels of DA have been shown in the striatum of both humans and animals (Hall et al., 1994; Jamal et al., 2022). Similarly, DOPAC, 3-MT, and HVA levels have also been found to be highest in that region of the brain (Hall et al., 1994; Jamal et al., 2022). Abnormalities in brain DA are associated with various neuropsychiatric disorders, including Parkinson's disease (PD), addiction, depression, and schizophrenia (Storga et al., 1996; Xu et al., 2022; Zhou et al., 2023). Tyrosine hydroxylase and dopa decarboxylase are involved in the synthesis of DA, while monoamine oxidase (MAO) and catechol-O-methyl transferase (COMT) are enzymes involved in the DA degradation. DA is synthesized in DA neurons and stored within vesicles, shielding it from MAO oxidation.

Two principal mechanisms are responsible for terminating DA action at synapses: DA is drawn back into the presynaptic neuron through reuptake and recycling, and it is sequentially transformed into the metabolites

Received 28 June 2024, accepted 6 August 2024

3,4-dihydroxyphenylacetic acid (DOPAC), 3-methoxytyramine (3-MT), and homovanillic acid (HVA) by COMT and/or MAO. Thus, the levels of DA metabolites reflect the activity of the dopaminergic system and provide valuable insights into enzyme function and potential abnormalities (Di Giulio et al., 1978; Ebinger, 1987). For example, changes in striatal DOPAC and 3-MT levels may reflect alterations in DA release and the firing rates of nigrostriatal dopaminergic neurons (Melamed et al., 1980; Karoum et al., 1994), assuming that DA is distributed equally among dopaminergic neurons. HVA, a primary end-product of DA metabolism, reflects central DA metabolism (Sternberg et al., 1983). Impairments in DA synthesis, storage, transportation, and metabolism have been linked to the neurodegeneration of dopaminergic neurons in PD models (Serra et al., 2002; Huang et al., 2022; Cramb et al., 2023).

COA-Cl (6-amino-2-chloro-9-[trans-trans-2,3bis(hydroxymethyl)cyclobutyl] purine) is a novel synthesized adenosine analog that structurally resembles adenosine (Sakakibara et al., 2015). When locally administered to rats, COA-Cl reduced perihematomal edema in intracerebral hemorrhage and improved neurological motor deficits by reducing oxidative stress (Lu et al., 2016). Additionally, COA-Cl has been found to induce angiogenesis and synaptogenesis *via* ERK activation in both *in vivo* and *in vitro* models (Tsukamoto et al., 2010; Okabe et al., 2013). Recent studies have shown that COA-Cl increases DA levels *in vivo* and *in vitro* (Jamal et al., 2019) and enhances spatial memory (Kishimoto et al., 2018). COA-Cl has also been shown to have beneficial effects on restoring motor function in rats (Sakamoto et al., 2021). Furthermore, COA-Cl promotes DA release through the phosphorylation of tyrosine hydroxylase in PC12 cells (Jamal et al., 2019). We hypothesize that COA-Cl increases DA levels, increasing metabolites in the mouse brain. To test this hypothesis, we performed a microdialysis study to determine the effects of COA-Cl (0.05–0.5 mM) perfused locally in the dorsal striatum on DA metabolism in mice. We used an *in vivo* reverse microdialysis technique coupled with high-performance liquid chromatography and an electrochemical detector (HPLC–ECD) to simultaneously measure DA and its metabolites DOPAC, 3-MT, and HVA. Clorgyline (4.0 mg/kg), an MAO oxidase inhibitor, was administered intraperitoneally (IP) to evaluate its role in COA-Cl-induced changes in DA metabolism.

METHODS

Animals

C57BL/6N mice were purchased from Japan SLC Inc. (Hamamatsu, Shizuoka). The mice were housed in groups of five and maintained at a temperature of 23 ± 1°C with 12 h of light exposure (07:00–19:00) per day. All experiments were conducted using male mice. Each mouse was 10–12 weeks in age and weighed 24–28 g. This study was conducted in accordance with the Guidelines for the Care and Use of Laboratory Animals at the Kagawa University Animal Investigation Committee (approval number 24621).

Experimental groups

The mice were divided into six experimental treatment groups: (a) Ringer's solution (control, n=5), (b) COA-Cl at 0.05 mM (n=5), (c) COA-Cl at 0.1 mM (n=5), (d) COA-Cl at 0.5 mM (n=5), (e) clorgyline at 4.0 mg/kg (n=5), and (f) COA-Cl at 0.1 mM + clorgyline (n=5). Local reverse microdialysis administration of COA-Cl was used to verify the direct effects of COA-Cl on brain DA release, although systemic administration would have been more clinically relevant. COA-Cl was obtained from Wako Pure Chemical Industry Ltd. (Osaka, Japan) as a commercial product (2Cl-C.OXT-A). It was synthesized as described previously (Tsukamoto et al., 2010). A stock solution of 2.0 mM COA-Cl was prepared in Ringer's solution. All prepared solutions were stored at 4°C until used. Clorgyline hydrochloride was purchased from Sigma (St. Louis, MO, USA), dissolved in 0.9% saline, and administered *via* IP injection in a 10 mL/kg volume.

Surgery

Each mouse was anesthetized *via* IP administration of a mixture of medetomidine hydrochloride (0.3 mg/kg), midazolam (4 mg/kg), and butorphanol tartrate (5 mg/kg) (Kirihara et al., 2013). The mice were then placed in a stereotaxic apparatus, and their skulls were exposed. A small hole was drilled using a dental drill, and a guide cannula was implanted into the dorsal striatum using the following coordinates according to the atlas of Paxinos and Franklin (2001): anterior, 0.2 mm to the bregma; lateral, 1.8 mm; depth, 2 mm. A dummy cannula was inserted, and the guide cannula was secured to the skull with dental cement anchored by a stainless-steel screw.

Reverse microdialysis

In vivo reverse microdialysis was carried out as previously described (Jamal et al., 2019). One day after post-operative recovery, the dummy cannula was replaced. The mice were anesthetized with diethyl ether,

and microdialysis probes were inserted into the dorsal striatum using guide cannulas. The probes were continuously perfused at a flow rate of 1 µL/min with Ringer's solution containing 147 mM NaCl, 4 mM KCl, and 2.25 mM $CaCl_2$ (pH 6.4), and dialysates were collected every 30 min using the HPLC–ECD system. After obtaining a stable 4-sample baseline of DA, the striatum was perfused with control or COA-Cl (0.05–0.5 mM) in Ringer's solution through the probe inlet over 180 min. The basal level (100%) was defined as the average output of four consecutive samples that did not differ by more than 4%. DA and its metabolite concentrations after COA-Cl administration were determined for each mouse as a percentage of the pre-COA-Cl baseline concentration.

HPLC-ECD conditions

To determine the *in vivo* concentrations of DA and its metabolites in the brain, we utilized an HPLC system equipped with an ECD-300 (Eicom, Japan). The main operating conditions for HPLC were as follows: column (EicompaK SC-5ODS; 3.0 mm × 150 mm), oven temperature of 25°C, detector, and oxidation potential of +750 mV *vs.* Ag/AgCl reference analytical electrode. The mobile phase consisted of 83% citrate–acetate buffer (pH 3.5) with 17% methanol, 190 mg/L sodium octane sulfonate, and 5 mg/L EDTA-2Na at a flow rate of 0.23 mL/min. The samples were analyzed for 30 min, and the chromatograms were recorded using PowerChrom software version 2.5 (eDAQ Pty Ltd., Densitone East, Australia). Stock standard solutions of 1.0 ng/µL DA and its metabolites were purchased from Eicom (Japan) and stored at 4°C until use.

Microdialysis probe recovery

In vitro probe recovery was measured to determine the concentrations of DA and its metabolites diffused into the dialysate fluid through the probe. Three microdialysis probes, each with a 2.0-mm length of dialysis tubing, were individually immersed in a 1.5-mL tube containing known concentrations (5.0–20 pg) of DA, DOPAC, 3-MT, and HVA in a 37°C water bath. Ringer's solution was perfused through the dialysis tube at a constant flow rate of 1.0 µL/min. Three consecutive dialysate samples were collected, with each sample collected for 25 min into a 0.4-mL tube on ice. Then, 10 µL of each sample was injected into the HPLC–ECD to determine the levels of DA and its metabolites. The recovery percentage was calculated as follows: probe recovery (Pr) = [(PAd / PAs) * 100], where PAd represents the peak area of the dialysate concentration, and PAs is the peak area of the standard solution concentration. The dialysate levels of DA and its metabolites were not corrected for the recovery.

Verification of probe and cannula placement

After the microdialysis experiments were completed, the mice were given a lethal dose of sodium pentobarbital (100 mg/kg) and euthanized. The brains were then removed, and the location of the dialysis probe in each brain was verified visually after sectioning. Only data from mice with the correct probe placement were included.

Statistical analysis

The average of the last four stable samples before treatment (less than 10% variation) was considered the control and defined as 100%. Data were analyzed using a two-way analysis of variance (ANOVA) with repeated measures over time and treatments (Ringer's solution, COA-Cl) as the independent factors. A *post hoc* Tukey-Kramer test was used for multiple comparisons. Individual comparisons were performed using Student's *t*-test. All analyses were conducted using SigmaPlot 14 software (Systat Software, Inc., Chicago, IL, USA).

RESULTS

Probe recovery

We determined the percentages of probe recoveries for three different concentrations (5.0, 10, and 20 pg) of DA, DOPAC, 3-MT, and HVA simultaneously, as shown in Table 1. Recovery tests were performed in triplicate at each concentration, with the results expressed as

Table 1. The *in vitro* probe recovery of DA and its metabolites DOPAC, 3-MT, and HVA at three different concentrations.

	Percentage recoveries		
	5.0 pg	10 pg	20 pg
DA	15.8 ± 2.7	15.6 ± 2.1	16.3 ± 1.1
DOPAC	20.3 ± 2.8	20.6 ± 1.6	21.5 ± 1.5
HVA	11.4 ± 2.0	19.2 ± 1.8	25.2 ± 2.0
3-MT	15.1 ± 2.2	16.3 ± 0.9	19.1 ± 1.8

DA, dopamine; DOPAC, dihydroxyphenylacetic acid; HVA, homovanillic acid; 3-MT, 3-methoxytyramine.

the recovery percentage of the DA and its metabolites standards. The recoveries of all compounds at the three concentrations are similar, except for HVA. There was a concentration-dependent increase in HVA recovery, suggesting that the recovery of HVA is linearly dependent on the concentration in the periprobe fluid.

Effects of COA-Cl on DA levels

Perfusion with COA-Cl produced a significant effect on DA levels (two-way repeated measures ANOVA, treatment effects: df 3,96; F=13.736; p<0.001; treatment × time interaction: df 3,18; F=8.797; p<0.001, Fig. 1A). *Post hoc* tests showed that treatment with 0.1 or 0.5 mM COA-Cl enhanced DA levels (Tukey-Kramer, p<0.05) compared to the control and 0.05 mM COA-Cl groups for a duration of 180 min post-COA-Cl perfusion. DA levels began to increase 60 min after COA-Cl perfusion and then gradually increased until 180 min. The elevation of DA was dose-dependent (p<0.05). Systemic administration of clorgyline alone or in combination with COA-Cl produced a significant effect on DA levels (treatment effects: df 3,96; F=7.233; p=0.003; treatment × time interaction: df 3,18; F=7.081; p<0.001, Fig. 1B). The *post hoc* Tukey-Kramer tests showed that clorgyline did not cause any statistically significant changes in DA levels when administered alone compared to the control group (p=0.995, Fig. 1B). However, when combined with COA-Cl, there was a similar increase in DA levels compared to clorgyline alone (p=0.014), indicating a COA-Cl effect.

Effects of COA-Cl on DOPAC and HVA levels

Perfusion with COA-Cl resulted in a significant effect on DOPAC (treatment effects: df 3,96; F=5.994; p=0.006; treatment × time interaction: df 3,18; F=3.493; p<0.001) and HVA (treatment effects: df 3,96; F=15.891; p<0.001; treatment × time interaction: df 3,18; F=15.479; p<0.001) levels compared to the control group (Fig. 2A and 3A). *Post hoc* analysis indicated that perfusion with 0.1 and 0.5 mM COA-Cl significantly increased HVA levels compared to the control and 0.05 mM COA-Cl groups (Tukey-Kramer, p<0.05). Furthermore, perfusion with 0.5 mM COA-Cl enhanced DOPAC levels compared to the control and 0.05 mM COA-Cl groups (Tukey-Kramer, p<0.05). The DOPAC and HVA levels began to increase 90 min after COA-Cl perfusion and remained elevated until the end of the 180-min period. This increase was dose-dependent (p<0.05). Treatment with clorgyline alone or in combination with COA-Cl also had a significant effect on DOPAC (treatment effects: df 3,96; F=14.329; p<0.001; treatment × time interaction: df 3,18; F=14.712; p<0.001, Fig. 2B) and HVA (treatment effects: df 3,96; F=13.280; p<0.001; treatment × time interaction: df 3,18; F=20.518; p<0.001, Fig. 3B) levels. The *post hoc* Tukey-Kramer tests revealed that clorgyline caused a significant decrease in DOPAC (p=0.019) and HVA levels (p=0.011) compared to the control group. Interestingly, when COA-Cl was combined with clorgyline, COA-Cl reversed the decrease in DOPAC (p=0.579) and HVA (p=0.049) levels caused by clorgyline, suggesting an activation of MAO by COA-Cl.

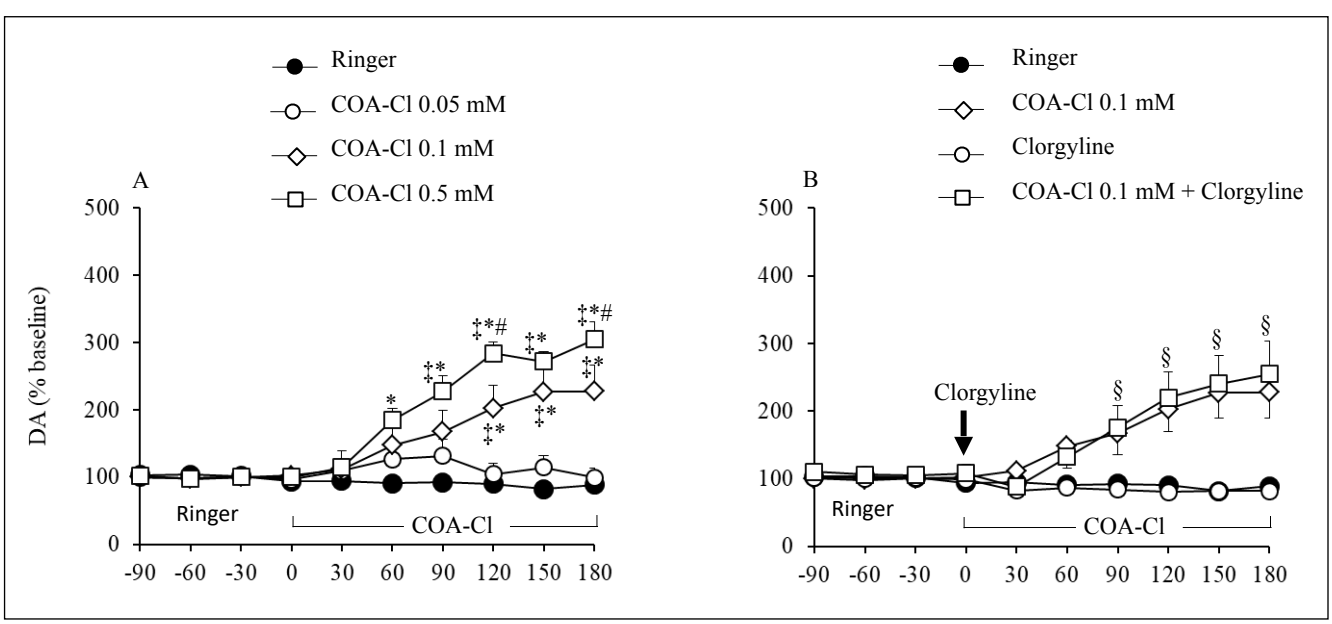

Fig. 1. Effect of COA-Cl (A) and clorgyline alone or in combination with COA-Cl (B) on DA levels. Values represent the mean ± SEM of independent determinations from 5 mice. *p<0.05 *versus* control; ‡p<*versus* 0.05 mM COA-Cl; #p<0.05 *versus* 0.1 mM COA-Cl; §p<0.05 *versus* clorgyline. DA, dopamine.

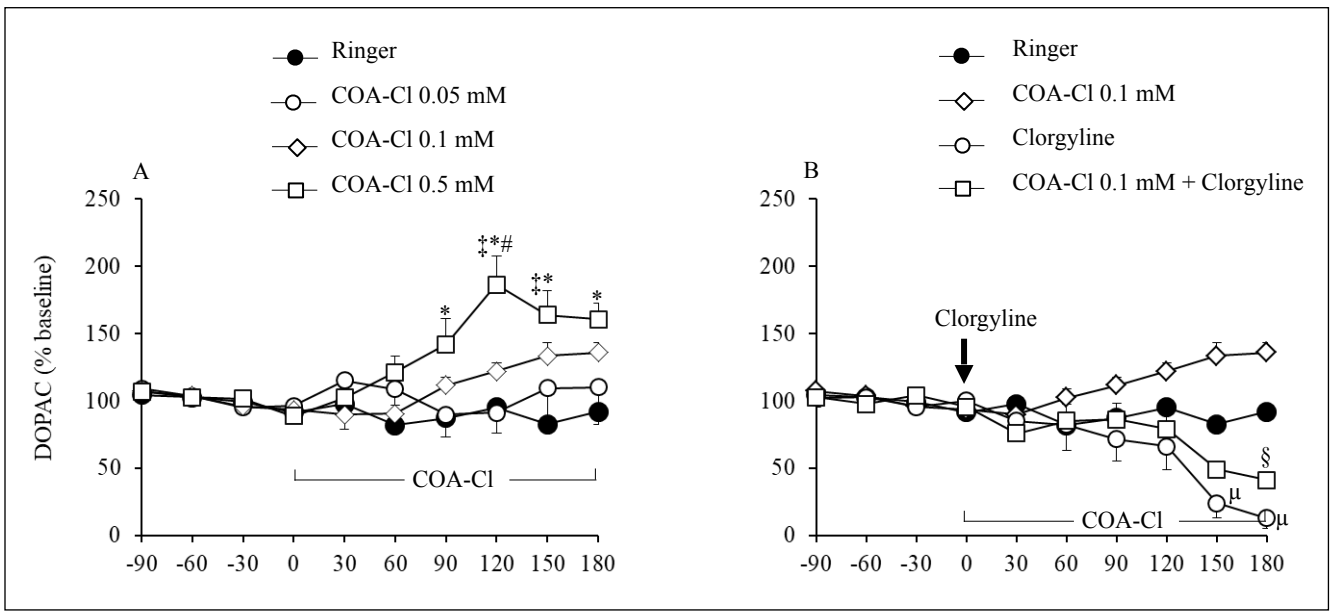

Fig. 2. Effect of COA-Cl (A) and clorgyline alone or in combination with COA-Cl (B) on DOPAC levels. Values represent the mean ± SEM of independent determinations from 5 mice. *p<0.05 versus control; ‡p<0.05 *versus* 0.05 mM COA-Cl; #p<0.05 versus 0.1 mM COA-Cl; µp<0.05 versus control; §p<0.05 *versus* clorgyline. DOPAC, dihydroxyphenylacetic acid.

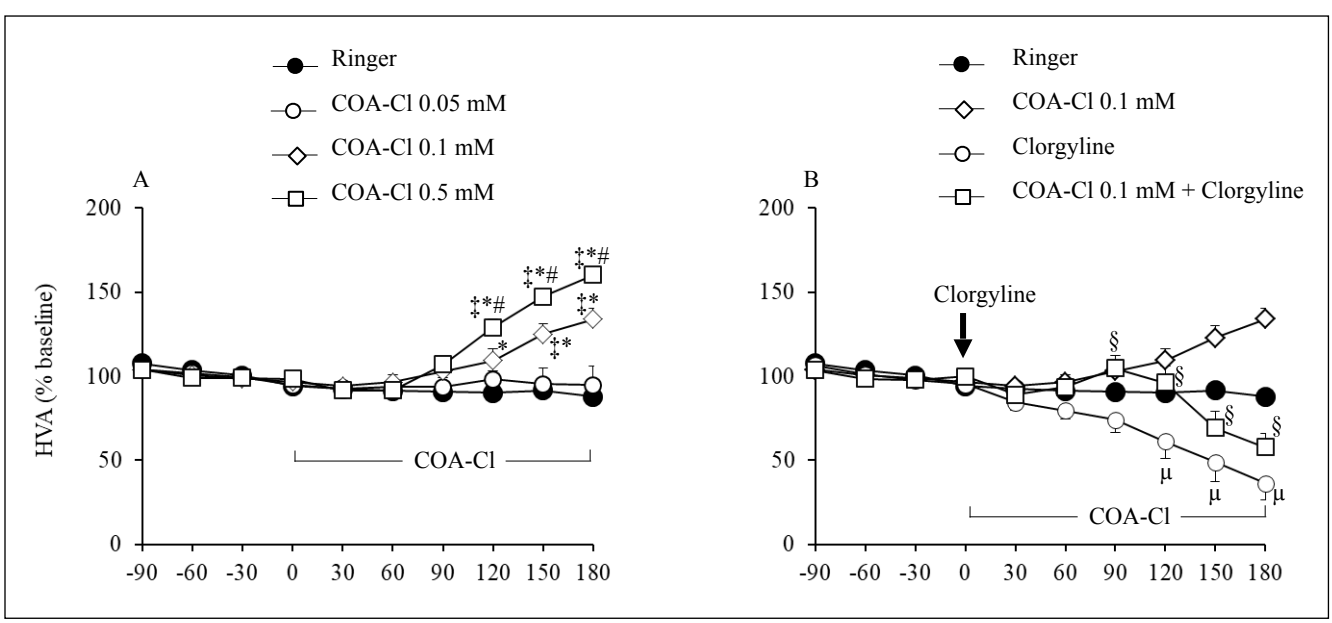

Fig. 3. Effect of COA-Cl (A) and clorgyline alone or in combination with COA-Cl (B) on HVA levels. Values represent the mean ± SEM of independent determinations from 5 mice. *p<0.05 *versus* control; ‡p<0.05 *versus* 0.05 mM COA-Cl; #p<0.05 *versus* 0.1 mM COA-Cl; µp<0.05 *versus* control; §p<0.05 *versus* clorgyline. HVA, homovanillic acid.

Effects of COA-Cl on 3-MT levels

Perfusion with COA-Cl had a significant effect on 3-MT levels (treatment effects: df 3,96; F=8.131; p=0.002; treatment × time interaction: df 3,18; F=9.198; p<0.001, Fig. 4A), paralleling the increase in DA levels. The *post hoc* Tukey-Kramer analysis revealed that perfusions with 0.5 mM COA-Cl enhanced 3-MT levels compared to the control and 0.05 COA-Cl groups (p<0.05). Clorgyline alone or in combination with COA-Cl also had

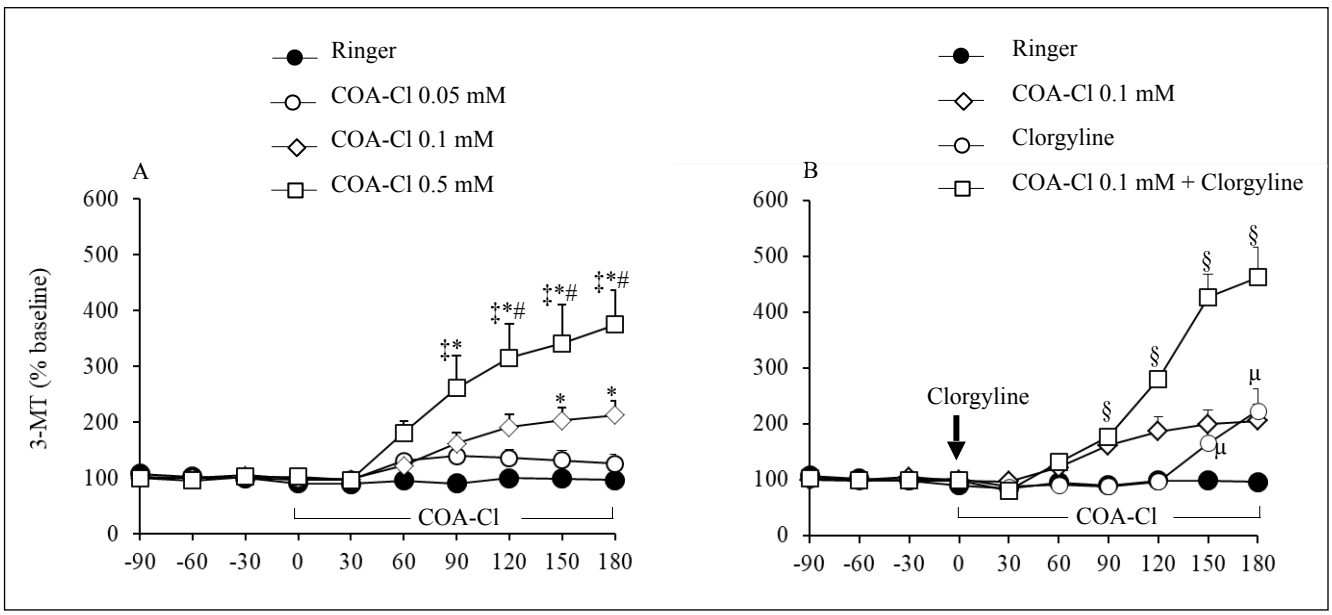

Fig. 4. Effect of COA-Cl (A) and clorgyline alone or in combination with COA-Cl (B) on 3-MT levels. Values represent the mean ± SEM of independent determinations from 5 mice. *p<0.05 *versus* control; ‡p<0.05 *versus* 0.05 mM COA-Cl; #p<0.05 *versus* 0.1 mM COA-Cl; µp<0.05 versus control; §p<0.05 *versus* clorgyline. 3-MT, 3-methoxytyramine.

a significant effect on 3-MT levels (treatment effects: df 3,96; F=26.747; p < 0.001; treatment × time interaction: df 3,18; F=17.930; p<0.001, Fig. 3B). The *post hoc* Tukey-Kramer tests showed that clorgyline produced a statistically significant increase in 3-MT levels when administered alone compared to the control group (p=0.045, Fig. 4B). Interestingly, when combined with COA-Cl, there was a further increase in 3-MT levels compared to clorgyline (p<0.001). Perfusion with 0.05 mM COA-Cl did not have significant effects on DA (p=0.750), DOPAC (p=0.809), HVA (p=0. 839), and 3-MT (p=0.812) levels compared to the control group.

DISCUSSION

We employed *in vivo* microdialysis to examine the local effects of COA-Cl perfusion on the extracellular levels of DA and its metabolites in the striatum of freely moving awake mice. This study is the first to examine the direct effect of COA-Cl on DA metabolism in the mouse brain. Our findings revealed that reverse microdialysis perfusion of COA-Cl (0.1 or 0.5 mM) significantly increased DA levels. These results are consistent with our previous study, which demonstrated a dose-dependent increase in dialysate DA following a moderate–high dose of COA-Cl (0.1–1.0 mM) in the mouse striatum (Jamal et al., 2019). We investigated whether the MAO inhibitor clorgyline could affect the extracellular levels of DA. Clorgyline alone did not produce any statistically significant changes in DA levels. However, when combined with COA-Cl (0.1 mM), there was a similar increase in DA levels compared to clorgyline alone, indicating an effect by COA-Cl. These data are consistent with a previous study that demonstrated clorgyline administration (0.5, 1.5, or 3 mg/kg) did not affect DA levels in the mouse cortex (Garcia-Miralles et al., 2016).

The modulation of extracellular DA levels occurs through two distinct mechanisms: release and uptake. The release of DA is influenced by DA synthesis and DA neuron activity, while the uptake is regulated by the dopamine transporter (DAT). Our findings support the former mechanism, as the increase in DA release induced by COA-Cl resulted in elevated extracellular DA levels. As further support, we previously found that COA-Cl (0.1 mM) increases DA release and tyrosine hydroxylase phosphorylation in the mouse striatum (Jamal et al., 2019). The reuptake of DA *via* the DAT serves as another mechanism through which DA is cleared from the synapse. Animals lacking DAT exhibit impaired reuptake of DA, leading to high and persistent levels of extracellular DA in the striatum, but significantly reduced total tissue DA levels (Jones et al., 1998; Efimova et al., 2016). Alternatively, we hypothesize that COA-Cl may inhibit DAT, leading to increased extracellular DA levels in the striatum. Further studies are required to validate this hypothesis. Together with our previous results (Jamal et al., 2019), these data support the notion that COA-Cl could po-

tentially elevate DA levels, making it a promising treatment strategy for PD with reduced DA levels.

DOPAC and HVA are the main degradation products of DA in the brains of both animals and humans (Espino et al., 1995; Ebinger et al., 1987; Yabe et al., 2009). The main pathophysiological characteristic of PD is the depletion of DA in the nigrostriatal system (Blesa & Przedborski, 2014; Grandi et al., 2018; Cramb et al., 2023). Consequently, concentrations of DA metabolites DOPAC, HVA, and 3-MT in the brain and cerebrospinal fluid are reduced in PD and mice treated with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (Eldrup et al, 1995; Serra et al., 2002; Morimoto et al., 2017). It is crucial to restore DA and its metabolites when treating PD. We hypothesize that COA-Cl can enhance DA metabolites DOPAC, HVA, and 3-MT in addition to DA. Therefore, our next goal was to measure the levels of DOPAC, 3-MT, and HVA following COA-Cl administration locally within the striatum of mice. The results revealed that local perfusion of 0.1 and 0.5 mM COA-Cl significantly increased HVA levels in the dialysates, where only 0.5 mM COA-Cl increased DOPAC levels. It has been shown that an increase in these two metabolites is likely a result of the increased DA level (Soares-da-Silva, 1987; Garrett & Soares-da-Silva, 1992). Because the levels of DA increased, the enhanced metabolism could be due to upregulated cytosolic DA resulting from an increased release rate, decreased reuptake, enhanced DA synthesis, or a combination of these factors. COA-Cl might activate dopaminergic neurons, inducing a release of DA locally within the striatum. This causes an increase in the extracellular DA level, resulting in increases in DOPAC and HVA levels.

MAO predominates in neural tissue that converts DA to DOPAC *via* oxidative deamination. The released DA is converted to HVA at extraneuronal sites through sequential metabolism by COMT and MAO. An increase in DOPAC and HVA levels may indicate enhanced DA metabolism due to the activation of MAO and COMT at dopaminergic terminals (Kaakkola & Wurtman, 1992). Therefore, we used the MAO inhibitor clorgyline alone or in combination with COA-Cl (0.1 mM) to determine whether COA-Cl influences the modification of MAO inhibitor action. Treatment with clorgyline alone significantly decreased DOPAC and HVA levels in the striatum, which is consistent with the findings of previous studies (Kaakkola & Wurtman, 1992; Wayment et al., 2001). Interestingly, when COA-Cl (0.1 mM) was combined with clorgyline, COA-Cl reversed the decrease in DOPAC and HVA levels caused by clorgyline, indicating an activation of MAO by COA-Cl. Thus, a moderate or high dose (0.1 or 0.5 mM) of COA-Cl might activate MAO, leading to increased DOPAC levels in the dialysates and, subsequently, higher levels of HVA in the mouse brain. The increased DOPAC and HVA levels together imply that COA-Cl can accelerate DA metabolism through increased DA release and/or activation of MAO in the striatum of mice.

3-MT is a major product of extracellular DA metabolism, formed by the direct catabolism of unused DA in the synaptic cleft by COMT (Männistö & Kaakkola, 1999; Myöhänen et al., 2010). Under physiological conditions, unlike other DA metabolites, such as DOPAC and HVA, 3-MT is present in the synaptic cleft at relatively low concentrations similar to those of the neurotransmitter itself and is a marker of DA release (Karoum et al., 1994). 3-MT can induce behavioral effects in a DA-independent manner, partially mediated by DA D1/5 receptor or the trace amine-associated receptor 1 (Nakazato & Akiyama, 2002; Sotnikova et al., 2010). Several studies have suggested that 3-MT is a sensitive indicator of DA concentrations in the synaptic cleft (Kehr, 1976; Wood & Altar, 1988; Brown et al., 1991; Karoum et al., 1994). The objective of this study was to test this hypothesis. We found that COA-Cl (0.1 or 0.5 mM) significantly increased 3-MT levels, paralleling the increase in DA levels. The increase in brain 3-MT levels following COA-Cl treatment could be due to increased DA release and/or COMT activation. Clorgyline alone also increased 3-MT levels due to the inhibition of MAO. Surprisingly, when combined with COA-Cl (0.1 mM), it further increased 3-MT, indicating a synergistic effect of COA-Cl on 3-MT levels. Numerous studies have explored DA release in various experimental models by measuring 3-MT levels in the animal brains after treatments with different MAO inhibitors (Kehr, 1976; 1981; Kumagae et al., 1991; Elverfors et al., 1997). The results demonstrated that the administration of MAO inhibitors significantly increased 3-MT levels. The observed increase in extracellular DA levels caused by COA-Cl supports the elevation in 3-MT levels. Overall, an increase in DA and its metabolites supports the idea that COA-Cl can enhance DA homeostasis in the mouse striatum.

CONCLUSION

Our results show that local exposure to moderate to high doses of COA-Cl (0.1 and 0.5 mM) increased extracellular levels of DA and its metabolites 3-MT and HVA in the dialysates of the mouse dorsal striatum. Perfusion with 0.5 mM of COA-Cl also increased DOPAC levels. The increased levels of DA after COA-Cl exposure may lead to an increase in DA and its metab-

olites' availability in the dopaminergic neurons. This is a new observation in the mouse brain, suggesting that COA-Cl acts directly in the brain to increase DA and its metabolites, providing new insights into the effects of COA-Cl on DA metabolism.

ACKNOWLEDGMENTS

This work was supported by a Grant-in-Aid for Scientific Research [Grant No. (c) 23K09766] from the Ministry of Education, Culture, Sports, Science and Technology, Japan.

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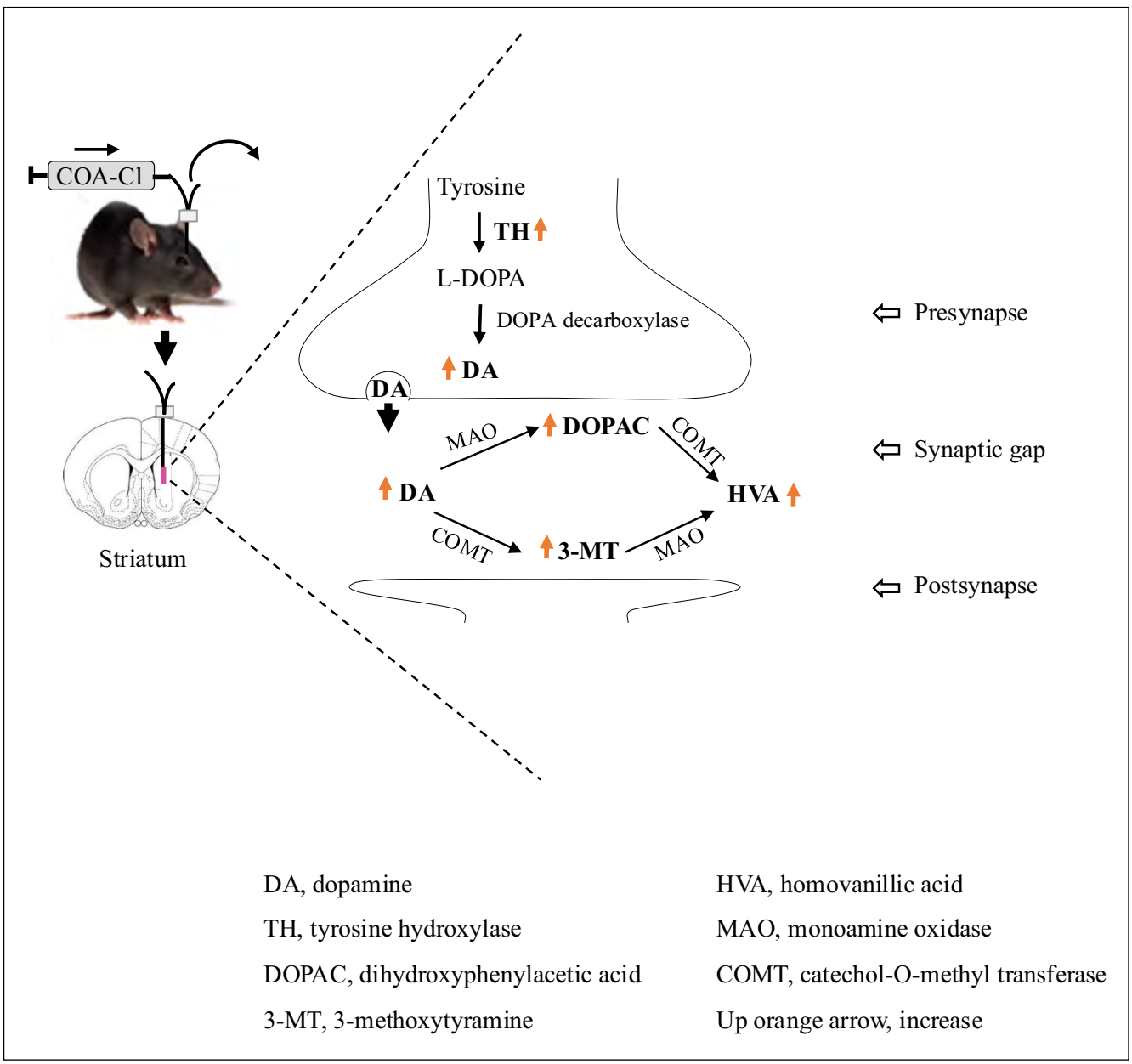
SUPPLEMENTARY MATERIALS



Supplementary Fig. 1. Graphical abstract.