The paradoxical effects of the dibenzodiazepine derivative, clozapine, on autonomic stimulating drug-induced salivary responses in mice

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Abstract: The effects of the dibenzodiazepine derivative, clozapine, on the salivary responses induced by autonomic stimulating drugs, and also on the levels of monoamines and ACh in the salivary glands and brains in mice were examined. The salivary responses induced by pilocarpine (0.8mg/kg, s.c.), phenylephrine (5mg/kg, s.c.), isoproterenol (0.4mg/kg, s.c.) and dopamine (10mg/kg, s.c.) reached a maximum level 20min after administration, but decreased thereafter and disappeared after 90min. Although clozapine (3, 10 and 30mg/kg) alone had no influence on salivary secretion in anaesthetized and non-anaesthetized mice, clozapine inhibited the pilocarpine-and dopamine-induced salivary responses in a dose-dependent manner. The phenylphrine-induced salivary response was increased with clozapine at 3mg/kg, and decreased at doses of 10 and 30mg/kg. However, clozapine had no influence on the isoproterenol-induced salivary response. The salivary responses induced by autonomic stimulating drugs showed the lowest level when clozapine (10mg/kg) was given 1 or 2 hours before the administrations. In mice treated with clozapine (10mg/kg), the ACh and DA levels decreased in the salivary glands, whereas the levels of monoamine related substances increased in the brain. These results suggest that the biphasic action of clozapine on the \(\alpha\)-adrenergic agonist-induced salivary response is related to the paradoxical symptoms of hypo- and hyper-salivation often observed in patients receiving clozapine.

抄録: ディベンゾジアゼピン誘導体 clozapine の自律神経作動薬による誘導唾液分泌反応、ならびに唾液腺と脳内モノアミンおよび ACh 含量に及ぼす影響についてマウスを用いて検討した。Pilocarpine (0.8mg/kg, s.c.), phenylephrine (5mg/kg, s.c.), isoproterenol (0.4mg/kg, s.c.) または dopamine (10mg/kg, s.c.) による唾液分泌反応は投与 20 分後に最大値を示し、その後減弱し、90 分後には消失した。Clozapine (3, 10, 30mg/kg) それは単独に無麻醉下または麻醉下のマウスの唾液分泌反応に影響を及ぼさなかったが、pilocarpine および dopamine の誘導唾液分泌反応を用量依存的に抑制し、phenylephrine の反応では clozapine の低用量で促進、高用量で抑制し、isoproterenol の反応には影響を及ぼさなかった。自律神経作動薬による唾液分泌反応は、clozapine が 1 または 2 時間前後に投与された場合に、その反応の抑制の程度は最も強かった。Clozapine (10mg/kg) 投与マウスにおいて、唾液腺内の ACh および DA 含量は低下したが、脳内のモノアミン関連物質含量は増大を示した。以上のことから、交感神経 \(\alpha\)-作動薬による誘導唾液分泌反応に対する clozapine の二相性作用が、clozapine を投与された患者において、しばしば観察される唾液分泌の減少あるいは過多の奇異な症状に関連性があるかもしれないことが示唆される。
Introduction

Clozapine of dibenzodiazepine derivatives is an atypical antipsychotic drug with pharmacological properties that are different from typical antipsychotic drugs such as chlorpromazine and haloperidol\textsuperscript{1-7}. Recent studies have shown that clozapine has an antagonistic effect on muscarinic acetylcholine receptors (mAChr)\textsuperscript{7,8}, \(\alpha\)-adrenergic receptors\textsuperscript{9}, serotonin receptors\textsuperscript{10}, dopamine receptors\textsuperscript{7,11,12} and histamine receptors\textsuperscript{13}. In addition, it was found that clozapine has no ability to induce the development of catalepsy, increase the levels of serum prolactin\textsuperscript{14}, or inhibit the stereotyped behaviors induced by apomorphine and amphetamine\textsuperscript{14}.

On the other hand, it is suggested that clozapine has a strong clinical antipsychotic effect on patients with schizophrenia\textsuperscript{3,6}, and that it is especially effective in improving the following symptoms: positive symptoms such as hallucinations and delusions of types found in patients with schizophrenia, and negative symptoms such as the showing of no emotions and secession from society\textsuperscript{3,6}. However, side effects such as sedation, tachycardia, hypotension, dizziness and agranulocytosis often develop in patients receiving clozapine\textsuperscript{1,15,16}, though extrapyramidal symptoms and tardive dyskinesia are absent\textsuperscript{1}.

Because it has an antagonistic effect on the cholinergic\textsuperscript{7,8,17} and adrenergic\textsuperscript{9} nervous systems, it is generally accepted that clozapine causes hyposalivation and dry mouth\textsuperscript{18}. It is also reported that in addition to this inhibitory effect, the agent induces ptyalism by stimulating salivary secretion\textsuperscript{6,19,20}. However, the mechanism of this paradoxical action (biphasic action: inhibition and stimulation) of clozapine on the response to salivary secretion is not entirely clear.

In the present study, to clarify the biphasic effects of clozapine on salivary secretion, the responses to salivation induced by autonomic stimulating drugs in mice receiving clozapine were examined pharmacologically. Based on the influence of clozapine on neurotransmitters, the levels of monoaminergic and cholinergic neurotransmitters in the salivary glands and brains were determined and discussed.

Materials and Methods

1. Animals

Male ddY mice, 5 weeks old, weighing 28\textendash 32 g, were purchased from the Shizuoka Laboratory Animal Center (Japan). Animals were housed, in groups of 10\textendash 12, in a room maintained under conditions of controlled lighting (a 12-hour light/dark cycle), temperature (23\textpm 2°C) and humidity (55\textpm 5%) with free access to standard chow (Oriental Yeast Co.) and water. All experiments using animals were performed according to the 'Guide for Care and Use of Laboratory Animals' at Iwate Medical University.

2. Drugs and administrations

Clozapine hydrochloride used in the present experiment was supplied from the SANDOZ Pharmaceutical Co. (Switzerland). Fig. 1 shows the chemical structure of clozapine.

Chemicals were purchased from the following sources: pilocarpine hydrochloride, dopamine (DA) hydrochloride, Na\textsubscript{2}EDTA, Na\textsubscript{2}HPO\textsubscript{4}, H\textsubscript{3}PO\textsubscript{4} and tween 80 from Kanto Chemicals; phenylephrine hydrochloride from Kowa Chemicals; isoproterenol hydrochloride from Nikken Chemicals; 3-methoxy-4-hydroxyphenylglycol (MHPG) hemipiperazine salt, and homovanillic acid (HVA) from Sigma Chemicals; 3, 4-dihydroxyphenylacetic acid (DOPAC), 5-hydroxyindoleacetic acid (5-HIAA), sodium heptanesulfonic acid, and sodium octanesulfonic acid from Aldrich Chemicals; norepinephrine (NE) bitartrate, serotonin (5-HT) creatine sulfate, acetyl-
choline (ACh) perchloride, tetraethylammonium chloride, 70% perchloride acid and urethane from Wako Pure Chemicals. Ethylhomocholine was synthesized in our laboratory according to the method of Potter et al.21. All other reagents and solvents were of special grade for use in high performance liquid chromatography (HPLC).

Clozapine was suspended in a 0.5% tween 80 solution immediately before every administration, and the dosages were 3, 10 and 30 mg/kg. In the experiments with mice pretreated with and without sialogogues, clozapine (3, 10 and 30 mg/kg) was injected 30 min before measuring salivary secretion. In the experiments on the influence of clozapine on the difference of administration time, clozapine (10 mg/kg) was injected 30 min, and 1, 2, 3, 5, 12 and 24 hours before the administration of sialogogues. In the experiments for measurement of the monoaminergic and cholinergic neurotransmitter contents in the salivary glands and brains of mice receiving clozapine (10 mg/kg), clozapine was injected 90 min before microwave irradiation (the time when indicated salivary responses were at the lowest level).

Sialogogues used in the present experiment were; pilocarpine (0.8 mg/kg) of partial muscarine agonists in the parasympathomimetics, phenylephrine (5 mg/kg) of α1-receptor agonists and isoproterenol (0.4 mg/kg) of β-receptor agonists in the sympathomimetics, and dopamine (10 mg/kg) of dopamine receptor agonists in the dopaminomimetics. Control animals were treated with 5 ml/kg of a 0.5% tween 80 solution. All agents were subcutaneously administered.

3. Measurement of saliva

Measurement of saliva was performed using the method of Murai et al.22, a somewhat modified version of Richter's method23. The mice were injected subcutaneously with urethane (1.0 g/kg body weight), and their heads and limbs were fixed with fine tape on a special fixing plate. Sialogogues were injected subcutaneously into the cervical back 30 min after the injection of urethane. The fixed mice were then immediately placed, face down on a sloping plastic plate covered with filter paper (TOYO Filter paper, No. 2), and moved to a fresh area on the filter paper every 10 min for 90 min.

The size of the saliva-stained spot on the filter was determined using a ScanJet IIC Scanner and an NIH image analyzer (Version 1.55, Wayre Rasband, NIH, USA) connected to a Macintosh computer (Centris 650). The amount of saliva secreted per unit time was evaluated. The values obtained over the 90-min observation period were compared with the control values.

Salivation experiments were carried out in a room with similar conditions of light, temperature and humidity to the breeding room. In addition, mice of similar age, body weight, and general condition were used in the experiments, and measurements were performed between 10:00 and 15:00 (once in the morning and once in the afternoon), taking the circadian rhythm in the salivation of mice into consideration.

4. Tissue preparation of salivary glands and brains of mice

Ninety min after the administration of either 10 mg/kg of clozapine or 5 ml/kg of a 0.5% tween 80 solution, each animal was irradiated by a microwave beam (5 kW, 0.7 sec., TMW-6402 C, Toshiba, Japan) focused on the head.

1) Tissue extraction

After microwave irradiation, the salivary glands of the right side were carefully removed and the submandibular, sublingual and parotid glands dissected out. The brain was removed from each mouse and dissected into four regions: cerebral cortex, hypothalamus, corpus striatum and hippocampus, according to a modified method of Glowinski and Iversen24. Isolated tissues from the salivary glands and brain were quickly frozen on dry ice, and then weighed and stored in a 1.5 ml Eppendorf microtube at −80°C until preparation.

2) Tissue preparation

After thawing, the isolated tissues were homogenized with an ultrasonic cell disruptor (Model 200, Branson, USA) in 300–400 μl of an ice-cooled 0.05 M perchloric acid solution containing 0.1 mM Na2EDTA for measurement of monoamines. However, for measurement of ACh, 750, 500, 750 and 300–400 μl of an
ice-cooled 0.1 M perchloric acid solution containing 0.1 mM Na2EDTA and 100 µM ethylhomocholine (internal standard for ACh assay) were added to homogenize the submandibular, sublingual and parotid glands and the brain, respectively.

The homogenates were centrifuged at 12,000 g for 20 min at 4°C. The clear supernatant was filtrated through a 0.45 µm filter (Type, HV, Nihon Millipore, Japan) and stored at −80°C until assay.

5. Chromatographic conditions for monoamines and related substances

Monoamines and related substances in the salivary glands and brain were measured by an HPLC-ECD system according to the method described by Murai et al.25,26.

The HPLC-ECD system consisted of a solvent delivery pump (Model L-6000, Hitachi, Japan), an analytical column (WH-C18, Hitachi, Japan), a guard column (Eicom Prepak, EICOM, Japan), a coulometric electrochemical detector (ESA, 5100 A, MA, USA), an integrator (Model C-R 6A, Shimadzu, Japan), and a column jacket connected to a thermostatic waterbath (Model UC-65, Tokyo Rika Kikai, Japan). The mobile phase was a 0.02 M acetic acid sodium/0.0125 M citric acid buffer (pH 4.0) containing 7.4% methanol, 0.045% heptanesulfonic sodium and 0.1 mM Na2EDTA. The conditions for measurement of the salivary glands and brain were: a flow-rate of 2.3 ml/min and a temperature of 40°C.

Substances measured were norepinephrine (NE) and its metabolite 3-methoxy-4-hydroxyphenylglycol (MHPG), dopamine (DA) and its metabolites 3, 4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), and serotonin (5-HT) and its metabolite 5-hydroxyindoleacetic acid (5-HIAA).

6. Chromatographic conditions for ACh

ACh in the salivary glands and brain were measured by an HPLC-ECD system according to the method described by Murai et al.25,26.

The HPLC-ECD system consisted of a solvent delivery pump (Model L-5000, Yanagimoto, Japan), an analytical column (AC-GEL, EICOM, Japan), an immobilized enzyme column (AC-Enzympak, EICOM, Japan), a catecholamine-trap column (CA-Trap, EICOM, Japan), a guard column (Eicom Prepak, EICOM, Japan), an electrochemical detector (Model VMD-5000, Yanagimoto, Japan), a platinum working electrode (WE-PT, +0.5 V, EICOM, Japan), an integrator (Model C-R 3 A, Shimadzu, Japan), and a column jacket connected to a thermostatic waterbath (Model UC-65, Tokyo Rika Kikai, Japan). The mobile phase was a 0.06 M phosphate buffer (pH 8.3) containing 0.01% tetraethylammonium chloride, 0.3% octanesulfonic acid sodium and 0.1 mM Na2EDTA. The conditions of measurement were set at a flow-rate of 1.9 ml/min with a temperature of 35°C for the salivary glands, and at the 2.0 ml/min and 33°C for the brain.

7. Statistical analysis

The results obtained were expressed as the mean values ± S. E. Statistical analysis was performed with ANOVA (one tailed) and Duncan new multiple range tests.

Results

1. Effect of clozapine alone on the response to salivary secretion in mice

In anaesthetized and non-anaesthetized mice, clozapine (3, 10 and 30 mg/kg) alone exerted no influence on salivary secretion.

2. Effects of clozapine on the pilocarpine-, phenylephrine-, isoproterenol- and dopamine-induced salivary responses

1) Pilocarpine-induced salivary response (Fig. 2)

Pilocarpine-induced salivation reached a maximum value 20 min after pilocarpine administration, decreased with time thereafter, and disappeared after 90 min. Such a salivary response was inhibited significantly depending on the dose of clozapine. However, maximum values of all responses were observed 20 min after pilocarpine administration. Furthermore, the total volume of saliva induced by pilocarpine treatment was reduced by 30% in mice receiving 3 mg/kg clozapine, 70% in those receiving 10 mg/kg clozapine and 75% in those receiving 30 mg/kg
2) Phenylephrine-induced salivary response (Fig. 3)

Phenylephrine-induced salivation reached a maximum value 20 min after phenylephrine administration, decreased with time thereafter, and disappeared after 90 min. The response to phenylephrine-induced salivation in mice treated with clozapine at 3 mg/kg was significantly decreased compared to the control 10 and 20 min after phenylephrine administration. This response reached a maximum value 30 min after phenylephrine administration, and decreased with time thereafter. However, the total volume of saliva during phenylephrine-induced salivation in mice treated with clozapine at 3 mg/kg increased significantly compared with the control. On the other hand, in mice receiving 10 and 30 mg/kg clozapine, the phenylephrine-induced salivary response decreased significantly, and the total volume of saliva was reduced by 70~80%.

3) Isoproterenol-induced salivary response (Fig. 4)

Isoproterenol-induced salivation reached a maximum value 20 min after isoproterenol administration, decreased with time thereafter, and disappeared after 90 min. No change in the salivary response and the total volume of saliva produced was observed in mice receiving 3, 10 and 30 mg/kg clozapine which was similar to the control.

4) Dopamine-induced salivary response (Fig. 5)

Dopamine-induced salivation reached a maximum value 20 min after dopamine administration, decreased with time thereafter, and disappeared after 90 min. All dopamine-induced salivary responses in mice treated with clozapine at 3, 10 and 30 mg/kg showed a significant decrease. However, maximum values of all responses were observed 20 min after dopamine administration. Furthermore, dopamine-induced salivation decreased significantly (by 40~55%) for all doses of clozapine.

3. Effects of pretreatment time of clozapine on the sialogogue-induced salivary responses

Salivation induced by sialogogues was investigated in mice that had 10 mg/kg clozapine administered at a fixed time.
Fig. 3 Effect of clozapine on the response to phenylephrine-induced salivation.
Phenylephrine (5 mg/kg, s.c.) was injected 30 min after administration of clozapine (3, 10 and 30 mg/kg, s.c.) or 0.5% tween 80 solution (control : 5 ml/kg, s.c.), and the volume of saliva produced was measured for 90 min. Values are the mean±S.E. (n=10). *** and **** : p<0.05, p<0.01 and p<0.001 compared to the control group (Duncan's test).

Fig. 4 Effect of clozapine on the response to isoproterenol-induced salivation.
Isoproterenol (0.4 mg/kg, s.c.) was injected 30 min after administration of clozapine (3, 10 and 30 mg/kg, s.c.) or 50% tween 80 solution (control : 5 ml/kg, s.c.), and the volume of saliva produced was measured for 90 min. Values are the mean±S.E. (n=10).
1) Response to pilocarpine-induced salivation

Fig. 6 (pilocarpine) shows the influence of time of pretreatment with clozapine on the response to salivation induced by pilocarpine. Salivation was reduced by 15~35% in mice receiving clozapine 30 min, and 1, 2, 3 and 5 hours prior to pilocarpine administration compared with the control. The smallest reduction, about 15%, was observed in mice receiving clozapine 2 hours before pilocarpine administration. However, in mice pretreated 12 hours before pilocarpine administration, the effect of clozapine on the salivary response was significantly weakened, and salivation was reduced by 81% compared with the control. Moreover, in mice receiving clozapine 24 hours before pilocarpine administration, the level of pilocarpine-induced salivation was similar to that of the control.

2) Response to phenylephrine-induced salivation

Fig. 6 (phenylephrine) shows the influence of time of pretreatment with clozapine on the response to phenylephrine-induced salivation. Salivation was reduced by 25~53% in mice receiving clozapine 30 min, and 1, 2 and 3 hours prior to phenylephrine administration compared with the control. However, in mice pretreated 5 and 12 hours before phenylephrine administration, the effect was significantly weakened, and the total amount of the phenylephrine-induced salivation was reduced by 90% compared with the control. Moreover, in mice receiving clozapine 24 hours before phenylephrine administration, the level of phenylephrine-induced salivation was similar to that of the control.

3) Response to isoproterenol-induced salivation

Pretreatment with clozapine had no affect on the response to isoproterenol-induced salivation (Fig. 6, isoproterenol).

4) Response to dopamine-induced salivation

Fig. 6 (dopamine) shows the influence of time of pretreatment with clozapine on the response to dopamine-induced salivation. Salivation was reduced by 20~53% in mice receiving clozapine 30 min, and 1, 2 and 3 hours prior to dopamine administration compared with the control. However, in mice receiving clozapine 5 and 12 hours before the administration, the effect of the salivary response was significantly weakened, and 24 hours before dopamine administration the level of dopamine-induced salivation was similar to that of the control.

![Graph showing effect of clozapine on dopamine-induced salivation](image)

Fig. 5 Effect of clozapine on the response to dopamine-induced salivation.

Dopamine (10 mg/kg, s. c.) was injected 30 min after administration of clozapine (3, 10 and 30 mg/kg, s. c.) or 0.5% tween 80 solution (control: 5 ml/kg, s. c.), and the volume of saliva produced was measured for 90 min. Values are the mean±S.E. (n=10). ** and ***: p<0.01 and p<0.001 compared to the control group (Duncan's test).
similar to that of the control.

4. Effects of clozapine on the levels of monoamines in the salivary glands

Measurement of levels of monoamines in the salivary glands was performed 90 min after administration of 10 mg/kg clozapine, under the same conditions as for the measurement of monoamine related substances in the salivary glands. The ACh levels in the submandibular, sublingual and parotid glands were significantly decreased compared with the control (Table 1).

6. Effects of clozapine on the levels of monoamines and its related substances, and of ACh in the brain

Measurements of brain neurotransmitters were performed under the same conditions as the salivary glands.

1) Levels of brain monoamine related substances (Table 2)

(1) Cerebral cortex: The levels of MHPG, DOPAC, HVA and 5-HT were significantly increased compared with those of the control.
Table 1  Levels of monoaminergic and cholinergic neurotransmitters in the salivary glands of mice treated with clozapine

<table>
<thead>
<tr>
<th>Submandibular gland</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10</td>
<td>3,390 ± 186</td>
<td>36 ± 3</td>
<td>481 ± 40</td>
</tr>
<tr>
<td>Clozapine 10 mg/kg</td>
<td>10</td>
<td>3,638 ± 162</td>
<td>26 ± 2*</td>
<td>541 ± 41</td>
</tr>
<tr>
<td>Sublingual gland</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>10</td>
<td>542 ± 27</td>
<td>17 ± 1</td>
<td>352 ± 24</td>
</tr>
<tr>
<td>Clozapine 10 mg/kg</td>
<td>10</td>
<td>527 ± 22</td>
<td>14 ± 1*</td>
<td>358 ± 25</td>
</tr>
<tr>
<td>Parotid gland</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>10</td>
<td>1,550 ± 65</td>
<td>33 ± 4</td>
<td>638 ± 38</td>
</tr>
<tr>
<td>Clozapine 10 mg/kg</td>
<td>10</td>
<td>1,494 ± 54</td>
<td>25 ± 1</td>
<td>636 ± 25</td>
</tr>
</tbody>
</table>

Mice were irradiated by a microwave beam 90 min after the administration of 0.5% tween 80 solution (control: 5 ml/kg, s.c.) or clozapine (10 mg/kg, s.c.), the salivary glands of the right side removed and submandibular, sublingual and parotid glands dissected. See the text for assay. Values are the mean ± S. E. (n = 10). * and **: p < 0.05 and p < 0.01 compared to the control group (Duncan’s test).

NE: norepinephrine. DA: dopamine. 5-HT: serotonin. ACh: acetylcholine.

Table 2  Levels of monoaminergic and cholinergic neurotransmitters in the brain of mice treated with clozapine

<table>
<thead>
<tr>
<th>Monoaminergic neurotransmitters</th>
<th>Cortex</th>
<th>Hypothalamus</th>
<th>Striatum</th>
<th>Hippocampus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Control</td>
<td>Control</td>
<td>Control</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CZP</td>
<td>CZP</td>
<td>CZP</td>
</tr>
<tr>
<td>NE</td>
<td>275±12</td>
<td>248±13</td>
<td>1,479±54</td>
<td>1,412±51</td>
</tr>
<tr>
<td>MHPG</td>
<td>41±1</td>
<td>113±5**</td>
<td>119±4</td>
<td>321±17**</td>
</tr>
<tr>
<td>DA</td>
<td>690±56</td>
<td>691±92</td>
<td>884±56</td>
<td>870±82</td>
</tr>
<tr>
<td>DOPAC</td>
<td>100±7</td>
<td>140±8**</td>
<td>264±11</td>
<td>352±23**</td>
</tr>
<tr>
<td>HVA</td>
<td>153±8</td>
<td>254±13**</td>
<td>270±15</td>
<td>413±33**</td>
</tr>
<tr>
<td>5-HT</td>
<td>686±25</td>
<td>1,022±37**</td>
<td>2,754±92</td>
<td>2,947±103</td>
</tr>
<tr>
<td>5-HIAA</td>
<td>136±4</td>
<td>188±7</td>
<td>655±25</td>
<td>691±23</td>
</tr>
</tbody>
</table>

Cholinergic neurotransmitters

<table>
<thead>
<tr>
<th></th>
<th>Cortex</th>
<th>Hypothalamus</th>
<th>Striatum</th>
<th>Hippocampus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Control</td>
<td>Control</td>
<td>Control</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CZP</td>
<td>CZP</td>
<td>CZP</td>
</tr>
<tr>
<td>ACh</td>
<td>4,380±175</td>
<td>4,205±292</td>
<td>4,906±204</td>
<td>5,037±175</td>
</tr>
</tbody>
</table>

Mice were irradiated by a microwave beam 90 min after the administration of 0.5% tween 80 solution (control: 5 ml/kg, s.c.) or clozapine (10 mg/kg, s.c.), the brain removed and the cerebral cortex, hypothalamus, corpus striatum and hippocampus dissected. See the text for the assay. Values are the mean ± S. E. (n = 10). * and **: p < 0.05 and p < 0.01 compared to the control group (Duncan’s test).

(2) Hypothalamus: The levels of MHPG, DOPAC and HVA were significantly increased compared with those of the control, but the levels of 5-HT and 5-HIAA showed no change.

(3) Corpus striatum: The levels of MHPG, DA, DOPAC, HVA, 5-HT and 5-HIAA were significantly increased compared with those of the control.

(4) Hippocampus: The levels of MHPG, DA, DOPAC, HVA and 5-HT were significantly increased compared with those of the control, but the 5-HIAA levels showed no change.

2) Levels of brain ACh

In the cerebral cortex, hypothalamus, corpus striatum and hippocampus the ACh levels showed no change (Table 2).

Discussion

Among patients receiving clozapine of dibenzodiazepine derivatives, the symptoms of dry mouth induced by hypo-salivation and/or ptyalism induced by hyper-salivation are often observed. To clarify these paradoxical symptoms, the effects of clozapine on the response to salivation induced by sialogogues were examined, because clozapine itself had no influence on salivation in anaesthetized or non-anaesthetized mice.

The response to salivation induced by pilocarpine, an mAChr agonist, reached a maximum value 20 min after administration, decreased with time thereafter, disappeared after 90 min, and was inhibited significantly depending on the dose of clozapine. These findings support previous results that clozapine blocks mAChr. However, Zom et al. reported that clozapine stimulated M1-receptors of a mAChr subtype, whereas Cawiey et al. reported that clozapine had no influence on mAChr. Thus, because clozapine displays various effects: stimulation, inhibition or no influence on mAChr its mode of action on the cholinergic nervous system is not uniform. However, in the present study, clozapine showed an anticholinergic effect on the pilocarpine-induced salivary response. Moreover, because extrapyramidal symptoms did not develop in patients treated with clozapine, it was considered that this was caused by the anticholinergic action of clozapine, the blockade of D2-receptors and the disruption of the monoaminergic nervous systems.

Salivation induced by phenylephrine, an α-adrenergic receptor agonist, and also isoproterenol, an β-adrenergic receptor agonist, were similar in terms of the time course of the response to pilocarpine-induced salivation, but the amount of saliva secreted was lower than that induced by pilocarpine. These responses reached a maximum value 20 min after administration of the agents, after which phenylephrine showed a rapid reduction and isoproterenol a slow reduction. Such a difference in the response patterns may be due to the different mode of action of both agents. The response to phenylephrine-induced salivation in mice treated with clozapine at 3 mg/kg increased significantly, and in mice treated with clozapine at 10 or 30 mg/kg decreased significantly, compared with non-treated mice. Thus, clozapine displays dosage-related biphasic activity: stimulation at low dosage and inhibition at high dosage in phenylephrine-induced salivary response. This supports the theory that clozapine has an inhibitory effect on α-adrenergic receptors. However, that a low dose of clozapine increases the phenylephrine-induced salivary response in mice is not clear from the present results. On the other hand, clozapine had no influence on the isoproterenol-induced salivary response.

The total amount of saliva secreted and the response pattern to dopamine were similar to those of phenylephrine-induced salivation. However, independent of the dosage, clozapine significantly inhibited the dopamine-induced salivary response compared with the control. These findings reflect the blocking of dopaminergic receptors by clozapine and are in agreement with reports by Meltzer and Ferris et al.

Furthermore, in the experiments on the effects of pretreatment time of clozapine on the response to salivation induced by pilocarpine, phenylephrine and dopamine, responses to salivation were of a similar pattern, except that induced by isoproterenol, i.e., when clozapine was administered 30 min, and 1, 2 and 3 hours before the sialogogues, the responses to salivation induced by the sialogogues decreased significant-
ly compared with the control. In addition, the levels of response were markedly lowered when clozapine was administered 1 and 2 hours before the sialogogues.

Moreover, the responses to phenylephrine- and dopamine-induced salivation in mice pretreated 5 hours before clozapine showed a pattern similar to the control, whereas the pilocarpine-induced salivary response was maintained in the inhibited state. The difference in these responses to salivation may be due to different interactions between clozapine and the sialogogues. In addition, in response to salivation induced by mAChr agonist, duration of inhibition was long, while in responses to salivation induced by \( \alpha \)-adrenergic and dopaminergic agonists, duration was short. However, clozapine had no influence on the response to salivation induced by \( \beta \)-adrenergic agonist.

It is well known that salivary secretion is mainly regulated by cholinergic and adrenergic nervous systems which are distributed to the salivary glands\(^\text{39}\). Thus, the levels of monoamine related substances and ACh, which influence the autonomic nervous systems, in salivary glands were determined in the present study. Clozapine significantly decreased the DA levels in the submandibular and sublingual glands of mice, but exerted no influence on the levels of NE and 5-HT in the submandibular, sublingual and parotid glands. Clozapine also significantly decreased ACh levels in the submandibular, sublingual and parotid glands.

Because clozapine is an atypical antipsychotic drug with pharmacological properties\(^\text{1–7}\), and differs from typical antipsychotic drugs such as chlorpromazine and haloperidol, and is effective in improving the positive and negative symptoms in patients with schizophrenia\(^\text{8–10}\), it is expected to prove a useful antipsychotic drug. In the present study, to compare the effect of clozapine on neurotransmitters in the peripheral and central nervous systems, the levels of monoamine-related substances (NE, MHPG, DOPAC, HVA, 5-HT and 5-HIAA) and ACh in the salivary glands and brains of mice were determined. The results showed that clozapine induced an increase in levels of brain monoamine-related substances but not in salivary glands. Moreover, clozapine induced no change in the ACh levels in any area of the brain, but induced a decrease in the salivary glands. These results suggest that there is a difference in the mode of action of clozapine on the neurotransmitters in the peripheral and central nervous system. However, because no paradoxical effects of clozapine on neurotransmitters in the peripheral and central nervous systems were observed, the relationship between the paradoxical effects of clozapine on salivary response and the changes of the neurotransmitters remains unclear.

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**References**

8) Miller, R. J. and Hiley, C. R.: Antimuscarine


of action cannot be explained by a direct block-
ade of postsynaptic dopaminergic receptors in
33) Putney, J. W. Jr., Weiss, S. J., Leslie, B. A. and
Marier, S. H.: Is calcium the final mediator of