Dopamine D$_1$ receptor antagonist inhibits swallowing reflex in guinea pigs

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Jia, Yu Xia, Kiyohisa Sekizawa, Takashi Ohhrui, Katsutoshi Nakayama, and Hidetada Sasaki. Dopamine D$_1$ receptor antagonist inhibits swallowing reflex in guinea pigs. Am. J. Physiol. 274 (Regulatory Integrative Comp. Physiol. 43): R76–R80, 1998.—To determine whether dopamine D$_1$ receptor antagonist impairs the swallowing reflex and reduces substance P (SP) in the peripheral organs, the swallowing reflex in terms of the number of swallows elicited by injections of three different volumes (0.2, 0.4, and 0.6 ml) of distilled water into the pharynx through a catheter was examined in anesthetized guinea pigs pretreated with Sch-23390. Animals were pretreated with either subcutaneous Sch-23390 (200 µg/kg) or a vehicle of Sch-23390 every 12 h for 7 days. The number of swallows was counted for submental electromyographic activity and visual observation of characteristic laryngeal movement. Injections of distilled water caused a volume-dependent increase in the number of swallows in animals without Sch-23390 treatment. Sch-23390 significantly decreased and exogenously administered SP increased the number of swallows elicited by all volumes of distilled water. FK-888 (10$^{-5}$ M, 1 ml), a specific inhibitor of the NK$_1$ receptor, reduced the number of swallows to a greater degree than Sch-23390.Sch-23390 significantly reduced SP content in the laryngeal and pharyngeal mucosa compared with control. These results suggest that inhibition of the dopamine D$_1$ receptor may impair the swallowing reflex and reduce SP content in the peripheral organs.

substance P; swallowing disorder; Parkinson’s disease; aspiration pneumonia

DOPAMINE IS THE PRINCIPAL neurotransmitter in four major neural systems in the brain (8, 15). Among them, the largest is the nigrostriatal pathway, which originates from dopamine-synthesizing neurons of the midbrain substantia nigra complex and innervates the dorsal striatum. Degeneration of this pathway leads to Parkinson’s disease (9), and an impaired dopamine metabolism is observed in patients with infarctions in the basal ganglia (10, 29). These patients frequently have swallowing disorders, which cause substantial morbidity and mortality due to aspiration pneumonia (21, 31).

With use of gene targeting technology, mice lacking the dopamine D$_1$ receptor (30) and dopamine synthesis (33) were created. These mutant mice exhibited abnormal motor activities, feeding problems, and reduced substance P (SP) immunoreactive nerve fibers in the striatum (30, 33). Kummer (14) showed that there are some neurons that are positive for tyrosine 3-hydroxylase but not for dopamine β-hydroxylase in the nodose ganglia, dorsal root ganglia, and sensory ganglia of the glossopharyngeal nerves in many species including guinea pigs (14). Tyrosine 3-hydroxylase is important in dopamine synthesis and therefore it is likely that neurons positive for this enzyme can produce dopamine. Therefore, dopamine signaling may regulate SP production in these ganglia where SP is synthesized and transported to the lung and the upper digestive tract (25). A previous study showed that depletion of SP by treatment with a large amount of capsaicin and a NK$_1$ receptor (SP receptor) antagonist impaired the swallowing reflex in guinea pigs (11). Taken together, these findings suggest that an inhibition of dopamine may cause a reduction of SP content in the larynx and pharynx where the swallowing reflex is triggered. To test this hypothesis, the effects of a specific inhibitor of dopamine D$_1$ receptor, Sch-23390 (30), on the swallowing reflex and SP content in the laryngeal and pharyngeal mucosa were investigated in guinea pigs.

METHODS

Hartley strain guinea pigs (Funabashi Farm, Shizuoka, J apan) weighing 500–650 g were studied. The animals were anesthetized intraperitoneally with ketamine (55 mg/kg) (11), and the hair around the neck was shaved. The animals were then laid in the supine position on a heated pad to maintain a rectal temperature of 37°C, and the four extremities were gently held with cords.

To measure the swallowing reflex, a catheter (OD 3 mm, Nelaton Catheter, Creat Medic, Yokohama, J apan) was inserted through the mouth so that the tip of the catheter lay in the pharynx (11). In each animal three different volumes of distilled water (0.2, 0.4, and 0.6 ml) were injected in a random sequence at intervals of 5 min. Three injections were performed for each volume of distilled water (11). The swallowing act was identified by submental electromyographic (EMG) activity and visual observation of the characteristic laryngeal movement (11, 23, 26). EMG activity was recorded from surface electrodes placed on the chin, and the resulting signals were filtered and amplified (San-ei Biophysiograph 180 System, Tokyo, J apan). The response to injections of distilled water was analyzed in terms of the number of swallows elicited (11, 22, 23, 26). The number of swallows was counted for 1 min after the injection of distilled water (11).

To examine whether dopamine D$_1$ receptors modulate the swallowing reflex, animals were treated with subcutaneous injections of a selective dopamine D$_1$ receptor antagonist Sch-23390 (200 µg/kg) every 12 h for 7 days (30). The control animals were also treated with the vehicle of Sch-23390 in a manner similar to that of Sch-23390 treatment. Responses to the injection of distilled water were studied in two separate groups of animals 8 h after the last injection of either Sch-23390 or vehicle: 1) animals without Sch-23390 treatment (control, n = 10) and 2) animals with Sch-23390 treatment (n = 10). To examine the acute effects of Sch-23390 on the swallowing reflex, animals (n = 10) were injected subcutaneously with Sch-23390 (200 µg/kg) and tested 15 min later (30).
To examine whether SP modulates the swallowing reflex, the effects of SP were tested in 10 animals without Sch-23390 treatment. SP was dissolved in distilled water, providing 10-fold incremental concentrations from $10^{-4}$ to $10^{-6}$ M. After the responses to three different volumes of distilled water were examined, the effects of SP on the swallowing reflex were studied. Injections of distilled water containing SP into the pharynx through the catheter were performed from the lower concentrations at intervals of 15 min. However, three different volumes of distilled water containing the same concentration of SP were injected in a random sequence at intervals of 5 min (11).

To determine the effect of SP on the time course of the swallowing reflex, the swallowing reflex with 0.4 ml of distilled water was measured at an interval of 3 min after the measurement of the swallowing reflex with distilled water containing SP ($0.4 \text{ ml, } 10^{-6} \text{ M}$) in 10 animals without Sch-23390 treatment.

To determine whether an endogenous SP mediates distilled water-induced swallowing, the effects of a specific NK1 receptor antagonist FK-888 (5) were measured in 10 animals without Sch-23390 treatment. The solution of FK-888 ($10^{-5}$ M, 1 ml) or the vehicle of FK-888 was injected into the pharynx through the catheter. Fifteen minutes later the animals were challenged by three different volumes of distilled water. The concentration of FK-888 used in the present study was maximally effective for inhibiting SP-induced cough in awake guinea pigs (28).

SP-like immunoreactivity was measured as described previously (32) in animals pretreated with and without Sch-23390 ($n = 10$ each). After the elimination of the blood, each of the laryngeal and pharyngeal mucosa pulled off by a fine forceps was minced and heated with a fivefold weight of water in a boiling-water bath (Digital Uni Ace UA-100, Eyela, Tokyo, Japan) for 3 min. After acidification with acetic acid on cooling, the suspension was centrifuged at 3,000 rpm for 20 min at 4°C. The supernatant was evaporated and lyophilized. Each of the lyophilized materials was dissolved in 3 M acetic acid (5 ml), and the insoluble material was removed by centrifugation. The solution was then submitted to gel filtration on a Sephadex G-25 (3 × 131 cm) using 3 M acetic acid as the eluant. Fractions of 10 ml each were collected, of which 30 to 45 were pooled, lyophilized, and assayed by radioimmunoassay for SP. The assay was performed according to the dextran-charcoal method. The standard diluent used was 0.01 M phosphate buffer (pH 7.4) containing 0.5% bovine serum albumin, 0.025 M EDTA, 0.14 M NaCl, and 0.1% dithiothreitol. Standard SP or unknown sample (0.1 ml), rabbit antiserum SP (Amersham, Arlington Heights, IL; final dilution 1:30,000–60,000; 0.1 ml), and $^{125}$I-labeled compound (Amersham 0.1 ml) were added to the standard diluent (0.4 ml) in each tube. The mixture was incubated at 4°C for 48 h, when dextran-coated charcoal suspended in 0.01 M phosphate buffer (1 ml) was added. After standing for 30 min, the mixture was centrifuged at 3,000 rpm for 15 min at 4°C. The supernatant was separated by decantation and counted. This antiserum showed no cross-reactivity with neurokinin A and bradykinin (20). SP content was expressed as ficoimole per milligram of protein. Protein concentrations were measured using the method of Lowry and co-workers (16).

The following drugs were used: SP (Sigma Chemicals, St. Louis, MO); ketamine hydrochloride (Sankyo, Tokyo, J apan). Sch-23390 and FK-888 were kindly donated by Research Biochemicals (Natick, MA) and Fujisawa Pharmaceutical (Osaka, J apan). FK-888 was dissolved in 100% ethanol to give a stock solution of $10^{-1}$ M, which was stored at 4°C. The stock solution was diluted in distilled water as necessary.

Results are reported as means ± SE. Statistical analysis was performed by one-way analysis of variance and Duncan's multiple-range test. Significance was accepted at $P < 0.05$.

**RESULTS**

It can be seen in Fig. 1 that injections of distilled water caused a volume-dependent increase in the number of swallows in animals without drug treatment. Chronic administration of Sch-23390 or pretreatment with FK-888 ($10^{-6}$ M) significantly decreased the number of swallows elicited by injections of three different volumes of distilled water; greater effects were observed in the latter. However, neither an acute administration of Sch-23390 (200 µg/kg, 15 min; Fig. 1) nor the vehicle of FK-888 (6.6 ± 0.7 in 0.2 ml, 7.8 ± 0.6 in 0.4 ml, and 9.2 ± 0.8 in 0.6 ml; $P > 0.20, n = 10$) significantly altered the number of swallows elicited by injections of all volumes of distilled water compared with that in animals without drug treatment.

The addition of SP into the distilled water showed a dose-dependent increase in the number of swallows compared with control values (Fig. 2A). SP ($10^{-6}$ M) increased the number of swallows during the first 6 min, and the number of swallows had returned to the baseline value 12 min after the administration of SP (Fig. 2B).

SP-like immunoreactivities were significantly lower in the laryngeal and pharyngeal mucosa in animals pretreated with Sch-23390 compared with those in animals without Sch-23390 treatment (Fig. 3).

**DISCUSSION**

Substance P, dynorphin, and enkephalin are the primary neuropeptides found in the striatal projection...
neurons that express dopamine receptors (6, 7). Mice lacking either the dopamine D1 receptor (30) or dopamine synthesis (33) are demonstrated to have reduced SP immunoreactivities in both the cell bodies of striatal neurons and their terminals in substantia nigra pars reticulata. These results suggest that dopamine signaling normally induces SP in the striatum.

The present study also shows that blockade of dopamine D1 receptors by Sch-23390 decreased SP content in the laryngeal and pharyngeal mucosa, suggesting that cell bodies and nerve terminals synthesizing and transporting SP to the peripheral organs may receive dopamine signaling. In fact, sympathetic nerve terminals that are positive for tyrosine 3-hydroxylase innervate nodose ganglia, dorsal root ganglia and sensory ganglia of the glossopharyngeal nerve of guinea pigs (14), major sites of SP synthesis in the lung, pharynx, and esophagus (25). Furthermore, exogenously applied SP enhanced the swallowing reflex, and a specific NK1 receptor antagonist FK-888 decreased the number of swallows in animals without drug treatment. Therefore, it is likely that an endogenous SP released by distilled water provokes swallowing actions in the present study. Thus a reduction of SP may be a mechanism responsible for the impaired swallowing reflex observed in animals pretreated with Sch-23390 as observed in guinea pigs pretreated with a large amount of systemic capsaicin (11).

However, it should be noted here that this study was conducted in nondecerebrated animals anesthetized with ketamine, one of the antagonists of N-methyl-D-aspartate receptors (1), which affect respiratory neurons and respiratory rhythmicity. Because central pattern generators for respiration and swallowing are closely interrelated (2), the frequency of swallowing reflex could be influenced by ketamine, although pharyngeal reflex is not abolished by the use of ketamine (18).

Coordinating the receptive fields for deglutition with the cranial nerves involves branches from three cranial nerves: 1) the trigeminal, 2) the glossopharyngeal, and 3) the vagus. The most effective receptor regions for the elicitation of the pharyngeal phase are innervated by fibers of the glossopharyngeal nerve, carried through the pharyngeal plexus and by the superior laryngeal nerve of vagus (19). Both the glossopharyngeal and superior laryngeal nerves have been shown to contain SP (24, 25). Shingai and co-workers (26) suggested that swallowing elicited by a small amount of water was mainly due to a chemical effect and not to a mechanical effect. Therefore, chemical irritation of the pharyngeal mucosa by distilled water may activate sensory nerves and release SP, with the result that the swallowing reflex is initiated by stimulation of the glossopharyngeal nerve.

Kummer (14) showed that nodose ganglia, dorsal root ganglia, and sensory ganglia of the glossopharyngeal nerve received some neurons that were positive for tyrosine 3-hydroxylase. Although tyrosine 3-hydroxylase is important in dopamine synthesis, this enzyme also takes part in the synthesis of other vasoactive amines, so immunostaining experiments shown by Kummer (14) are not specific for dopamine. Therefore,
there may be an alternative explanation for the reduced swallowing reflex observed in animals pretreated with Sch-23390. It is well known that dopamine agonist treatments in the rat bring about a heightened striosomal expression of SP, and both dopamine D₁ and D₂ antagonists decrease it (7). The high density of SP in the brain is found in the substantia nigra, where the peptide is present in fibers and terminals and can be released by depolarization in a calcium-dependent manner (17). Electrophoretic administration of SP into single neurons in the substantia nigra induces a prolonged increase in the neuronal activity (25). Therefore, the effect of Sch-23390 might have been in the basal ganglia and relayed to the peripheral nervous system.

Parkinson’s disease is characterized by degeneration in the pigmented nuclei of the central nervous system. However, gastrointestinal dysfunction (i.e., swallowing disorder and constipation) is a well-recognized feature of Parkinson’s disease (4). Singaram and co-workers (27) demonstrated a dopaminergic defect of the enteric nervous system in Parkinson’s disease patients with chronic constipation. Mice lacking the dopamine D₁ receptor and those treated with Sch-23390 showed abnormal motor activities and feeding problems (30). Furthermore, injections of capsaicin solution into the pharyngeal region stimulated the swallowing reflex, and the SP concentration in sputum, induced by hyper tonic saline, was reduced in patients with an impaired swallowing reflex (3, 20). Finally, an injection of levodopa improved the swallowing reflex in patients with aspiration pneumonia (12). These findings imply an important role of dopamine and SP in patients with swallowing disorders.

Because the act of swallowing is a fundamental defense mechanism against aspiration of oropharyngeal contents into the respiratory tract, impairment of the swallowing reflex is one of the major reasons for the development of aspiration pneumonia (13). The present study suggests that systemic treatment with dopamine D₁ receptor antagonist reduces SP in the laryngeal and pharyngeal mucosa, with the result that the swallowing reflex may be impaired in the guinea pigs. Such phenomenon may be relevant to patients such as those with Parkinson’s disease.

Perspectives

Swallowing disorder is a well-recognized feature of Parkinson’s disease. The characteristic neurological signs of the disease result from degeneration in the pigmented nuclei of the central nervous system and selective depletion of dopamine occurs in the neostriatum. It is now known that dopamine regulates SP production in the striatum. SP also exists in the endings of peripheral sensory nerves. On stimulation, SP is released from sensory nerves both at the site of stimulation and at nearby areas that are also innervated by this nerve. This local release of SP may participate in the swallowing reflex induced by distilled water injected into the pharynx. Chronic treatment with a dopamine D₁ receptor antagonist decreases both the number of swallows elicited by distilled water and the amount of SP in the laryngeal and pharyngeal mucosa. Although precise mechanisms for an impaired swallowing reflex caused by an inhibition of dopamine activity are uncertain, it is tempting to speculate on the possible role of peripheral SP regulated by dopamine-containing neurons to induce reflex swallowing.

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