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Effects of Diphenhydramine, Naphazoline and m-Amino-α(1-Aminoethyl)Benzyl Alcohol Dihydrochloride on the Nasal Mucosa Determined by Impedance Method: a Simple Method for Evaluation of Nasal Decongestant

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Abstract. A simple, new method was devised for evaluating nasal decongestants. In anesthetized dogs, two needle electrodes were inserted bilaterally into the superficial mucosa of the nasal wings. An impedance plethysmograph provided with a DC output was used to measure impedance between the two electrodes. When 1% histamine solution was sprayed into a nostril focusing on the inside mucosa of a nasal wing, impedance decreased markedly and thereafter recovered to a control level within 1–1.5 h. Comparable responses were obtained when the same solution was sprayed into the opposite nostril. The drugs to be tested were administered intravenously or topically between these two histamine applications. Intravenous administrations of diphenhydramine (0.5 mg/kg) and m-amino-α(1-aminoethyl)benzyl alcohol dihydrochloride (0.1 mg/kg) inhibited the histamine effect completely. Pretreatment with naphazoline administered topically also inhibited impedance changes caused by histamine application. Local applications of acetylcholine (10%) and bradykinin (0.1%) did not change nasal impedance significantly in any instances.

So far, vascular reactions in the nasal mucosa have been determined mainly by two methods, namely, pressure rhinometry (12, 18) and photoelectric plethysmography (5). In the former, the size of the nasal airway can be measured by the resistance to the airflow through the nostril. In the latter, the apparatus is applied to the nasal septum and variations in the blood content of the transilluminated tissue can be recorded. Although both can reflect hemodynamic conditions in the nasal mucosa even during prolonged observation, a considerable mass must be placed in the nasal cavity and this hinders the physio-

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Fig. 1. Measurement of nasal impedance. An anesthetized dog was tied loosely to the table in supine position. Two needle electrodes were inserted bilaterally under the mucosa of nasal wings.

Logical airflow which is needed to maintain the normal humidity of inhaled air, and under such circumstances the blood flow in the nasal mucosa may be significantly affected. Furthermore, in photoelectric plethysmography mechanical attachments must be constructed just as rhinometry requires various pieces of equipment to maintain humidity of the oxygen and constancy of the airflow. The present experiment was conducted to devise a new method which is more convenient but still permits objective evaluation of nasal decongestants.

Methods

Measurement of Nasal Impedance

Adult dogs, weighing from 5 to 15 kg, were anesthetized by intravenous injection of nembutal (30 mg/kg), and two pointed stainless steel electrodes were inserted bilaterally under the superficial mucosa of the nasal wings, keeping the needle axis parallel to that of the nose (fig. 1). Impedance was measured with an AC impedance bridge of 10 kHz and 100 mV bridge voltage. A block diagram of the circuit is given in figure 2. Two arms of the symmetrical, equal ratio bridge consisted of 100 Ω resistors. The third arm consisted of the tissue to be measured and the fourth of a resistance and capacitance box in parallel (1, 3). The bridge output was amplified and demodulated; thereafter only the DC component of the signal was fed to the high-gain DC amplifier.
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Fig. 2. Electrical circuit for impedance measurement. Magnitude of measuring current was adjusted to 10 mV peak to peak by $R_1$, $R_2$; 100 $\Omega$ each. $R_3$, $R_4$; 10 k$\Omega$ variable. $R_5$, $R_6$; 10, 20, 30 $\Omega$, respectively and these are for calibration. Bridge output was amplified by AC-AMP. OSC = Oscillator circuit (10 kHz); DEM = demodulating circuit.

Along with the experiment using the impedance bridge, nasal impedance was also measured by impedance plethysmograph (IMP-26, Nihon Koden Co.). In this instrument, a measuring current of 1 mA with bridge frequency of 50 kHz was employed. The block diagram and principles for impedance measurement are given in figure 3. When impedance changes due to histamine applications were recorded from the same dog by these two instruments, a considerable similarity in responses was obtained. In the present experiment, IMP-26 was mostly used for impedance measurement. After 20-30 min with the electrodes inserted, the baseline of nasal impedance became stable and thereafter 1% histamine solution or other phlogistics were sprayed on the inside mucosa of the nasal wings under a pressure of 360 mm Hg. At that time, care was taken to prevent inhalation of the agents into the respiratory organs.

Measurement of Arterial Blood Pressure
A small branch of the left femoral artery was cannulated to record systemic blood pressure. The devices used consisted of a pressure transducer (Statham p23 Db), carrier amplifier (RP-5, Nihon Koden) and pen-writing recorder (RJG-400, Nihon Koden).

Preparation of Histological Specimens
When the impedance diminished to a minimum after histamine application, the affected area of the nasal mucosa was excised and histological examination performed to study the correlation between impedance decrease and morphological changes. At the same time, the corresponding mucosa of the opposite nostril was also excised and both were
Fig. 3. Block diagram of IMP-26. The output of a 50-kHz oscillator was divided into two parts: one was applied to the animal body through electrodes and the other to a variable resistor (VR). Signal voltage produced between the electrodes was amplified after passing through a transformer and demodulated. Similar demodulation was also effected on that passing through the variable resistor. Since these two signals are out of phase, addition of the two eliminated a shift of DC level and formed a null circuit. The last stage of amplification was made with DC-AMP.

compared. The excised tissues were fixed in 10% formaline solution (pH 7.4) and thereafter, washing, dehydration with alcohol, clearing, and embedding were performed in succession. Sliced specimens were stained with a hematoxylin and eosin solution.

Phlogistics

As possible phlogistics, to provoke symptoms similar to those of nasal allergy in the nasal mucosa of the dog, histamine, acetylcholine and bradykinin were chosen in the present experiment, since they are all vasoactive (9, 15, 20) and their presence in the nasal mucosa has been reported previously (2, 4, 14). Among these agents, histamine and acetylcholine have been used for provocation of nasal allergy in human beings (7, 13) and in the present experiment both were tested in various concentrations. Bradykinin was used at 0.1% which was the highest concentration available to us at that time. All these agents were dissolved in 0.9% saline and the solutions were usually adjusted to pH 7.0.

Evaluation of Nasal Decongestant

The evaluation of nasal decongestant was done as follows: (1) firstly, impedance reduction due to the spraying of 1% histamine solution into the right nostril was confirmed; (2) secondly, after sufficient time had elapsed, at least 1 h after complete recovery from the histamine effect, the test drug was given either intravenously or topically as in the case of naphazoline; (3) thirdly, 1% histamine solution was sprayed into the left nostril 10–20 min after drug administration.

Test drugs were evaluated by comparison of impedance tracings obtained before and after drug administration. When incomplete inhibition was observed, the doses of the drugs being tested were increased in subsequent experiments until complete inhibition was achieved. As explained later, in many cases the effects of the two histamine applications
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Results

Impedance Change after Histamine Spray

When nasal impedance was recorded, small fluctuations associated with respiratory movements were seen in all instances. To clarify the reason for this rhythmical change in impedance, phase relationship between the two impedance tracings taken simultaneously from the nose and thorax were compared as shown in figure 4. To obtain tracings from the thorax, two needle electrodes were inserted subcutaneously into the lower thorax with symmetrical apposition and impedance was measured in the same way as in the nose. Impedance increased at inspiration and decreased at expiration. As is apparent from the figure, both were almost in phase even though impedance waves taken from the nasal wings slightly preceded those of the thorax. To obtain another clue for elucidation of the small fluctuations, forced respiration was carried out after tracheal intubation with or without immobilization. As the spontaneous respiration continued during tracheal intubation, small waves in the nasal impedance were present as seen before intubation. When artificial respiration was superimposed upon spontaneous respiration, fluctuations were still observed. However, when dogs were immobilized by intravenous injection of succinylcholine...
(0.5 mg/kg), such fluctuations disappeared completely. From these findings it is clear that the small waves in the nasal impedance were derived from the movements of the nasal wings in spontaneous respiration.

Shortly after the electrodes were inserted into the mucosa of the nasal wings, the baseline of impedance started to increase and this continued for 20–40 min. This may be a transient phenomenon which arises at the boundary between electrodes and the tissue (17) and goes on until a steady state of DC-contact polarization potential is reached.

When the baseline of nasal impedance became stable, 1% histamine solution was sprayed four times into a nasal cavity. As shown in figure 5, after 2–3 min of histamine application, the nasal impedance began slowly and progressively to decrease until the minimum was attained 30–40 min later. At that time an appreciable amount of swelling in the inferior turbinate mucosa was noticed. After the nasal impedance reached the minimum, it began to increase again and this was followed by gradual elevation. The baseline of nasal impedance returned to the control level about 1.5 h after histamine application. Impedance changes were reasonably consistent in all of the 35 experiments. The magnitudes of actual impedance changes represented in terms of ohms and the durations required to reach the minimum which elapsed before termination of the response were determined for all histamine tracings. The mean of impedance reduction in 35 experiments was $18 \pm 1.9 \Omega$ (SE) and the mean durations required for reaching the minimum and for the whole response were $20 \pm 2.0$ (SE) and $76 \pm 4.5$ min (SE), respectively. Some correlation between the magnitudes of impedance change and the length of each of the durations was noticed: the larger the impedance reduction, the longer the duration.

In some cases, artificial deflections of the baseline towards an impedance decrease were observed at the time of spraying. However, even in such cases the
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Fig. 6. Comparison of impedance changes induced by histamine applications into bilateral nasal cavities of the same dog. At arrows, 1% histamine solutions (H) were sprayed four times. Between histamine applications a 2-hour interval was interposed.

impedance diminished further from the deflected baseline (fig. 11) and the subsequent responses were analogous to those obtained when proceeding without abrupt deflection. To confirm the uniformity of responses obtained from the two nostrils, which is essential for correct drug evaluation, a comparison of the two impedance changes induced by histamine applications into the two nasal cavities of the same dog was made first without drug administration. Histamine application was repeated after 3 h in each of 10 dogs. When histamine application was repeated after a 3-hour interval in each of the 10 dogs, the responses were similar both in magnitudes and durations as shown in figure 6. The mean of impedance reduction in 10 experiments after histamine application into the right nasal cavity was $20 \pm 3.4 \, \Omega$ (SE), and that of the left nostril was $22 \pm 3.8 \, \Omega$ (SE). The periods that were required to reach the minimum impedance and that were necessary to terminate the whole response were $20 \pm 3.4$ (SE) and $74 \pm 5.8$ min (SE) for the right nasal application, and $18 \pm 2.5$ (SE) and $83 \pm 7.4$ min (SE) for the left. The difference in the corresponding values in each of the parameters was not significant ($p < 0.01$). The magnitudes of the responses obtained from both sides were parallel. However, in a few cases some differences in magnitude between the two responses were noticed; for example, sometimes the effects of the earlier applications were somewhat greater than those of the following or vice versa. When histamine was applied in lower concentrations such as 0.5 or 0.1%, impedance changes were attenuated to various extents, even though some dose-response relationship remained. The durations of impedance changes also varied markedly, taking from 10 to 50 min to complete the whole response.

The pH of 1% histamine solution used in this experiment was 5.0. When this was adjusted to 7.4 with $0.01 \, N$ NaOH and sprayed into the nasal cavity, the impedance changes did not significantly differ from those of pH 5.0. A saline
solution of pH 5.0 or less, such as 4.0 or 3.0 was sprayed into the nasal cavities to see whether pH itself affects nasal impedance. However, except for a solution of pH 3.0, no noticeable change was observed.

In the histological specimens, taken from the affected area at the minimum impedance, a marked edema was easily observed in the lamina propria, with intracellular spaces having become wide and the connective tissue fibers swollen. Also, a remarkable dilatation of the small vessels was noticed.

**Impedance Changes due to Acetylcholine and Bradykinin**

When 10% acetylcholine was sprayed into a nasal cavity four times in each of 5 dogs, no significant change in nasal impedance was provoked in any of those dogs (fig. 7). As a preliminary to this experiment, acetylcholine spray was commenced from a 0.1% solution and thereafter, the concentration was increased to 10%. However, no noticeable change was observed in any of those experiments. Figure 7 also shows the impedance change due to 0.1% bradykinin spraying. A very slight decrease in nasal impedance was seen after an initial artifact. However, this decrease returned to the control level within a few minutes.

To provoke a symptom similar to nasal allergy, histamine spray has been frequently used in the clinical field (7). In this connection, it has been reported that when acetylcholine was used to provoke nasal allergy in the same manner, the effect was somewhat less than that of histamine (13) and this coincides with the result of the present experiment.

**Effect of Antihistamine**

When a characteristic impedance decrease due to histamine spray into a nasal cavity was observed, 0.5 mg/kg of diphenhydramine was given intravenously 2 h after the impedance drop returned to control level. 20 min after the injection, histamine was sprayed into the opposite nasal cavity. The treatment with diphenhydramine abolished the impedance change due to the subsequent histamine application in all of the three experiments (fig. 8). However, in a dose of less than 0.5 mg/kg, the inhibition of histamine effect was incomplete.

**Effect of Naphazoline Spray**

**Impedance change due to naphazoline alone.** When 0.05% naphazoline was sprayed several times into a nasal cavity no noticeable change in impedance was produced. Even when the experiments were carried out with 0.1 or 0.5% naphazoline, no significant change in nasal impedance was seen in any of the three experiments as shown in figure 9.

**Impedance changes due to the combination of histamine and naphazoline.** Impedance change after the spraying of a test solution composed of 10% histamine and 0.05% naphazoline in the ratio of 1:9 is shown in figure 10. In this case, impedance change was noticeably less than that of histamine alone both in
Fig. 7. Local applications of acetylcholine and bradykinin. At arrows, 10% acetylcholine (A) and 0.1% bradykinin (B) were sprayed. In no case was acetylcholine application effective. Bradykinin reduced nasal impedance slightly for only a few minutes.

Fig. 8. Inhibitory effect of diphenhydramine on impedance change due to histamine application. Intravenous injection of 0.5 mg/kg diphenhydramine (D) completely inhibited impedance change due to application of 0.1% histamine (H).

Fig. 9. Impedance changes due to naphazoline applications in various concentrations. At arrows, 0.05, 0.1 and 0.5% naphazoline (N) were applied to the nasal mucosa of dogs. In no instance was any remarkable change in nasal impedance observed.
Fig. 10. Impedance changes due to the combinations of histamine and naphazoline. Both were combined in the ratio of naphazoline 9–10 % histamine 1 (vol/vol). When 0.05 % naphazoline (N) was mixed with histamine (H), some inhibitory effect was noticed, but it was incomplete. However, when the concentrations of naphazoline were higher than 0.1 %, complete inhibition was effected.

the magnitude and duration. When a 0.05 % naphazoline solution was increased to either a 0.1 or 0.5 % solution in similar experiments, impedance tracings were entirely flat.

**Effect of naphazoline pretreatment on histamine application.** In this experiment, 0.05 % naphazoline was sprayed several times into a nostril prior to 1 % histamine application. Both were made into the same nostril at a 10-min interval. Impedance change after the histamine application was less than that after a single histamine application (fig. 11). Similar experiments were performed after pretreatment with both 0.1 and 0.5 % naphazoline solutions. In those cases, impedance changes due to histamine were completely inhibited.

**Effect of m-Amino-α(1-Aminoethyl)Benzy1 Alcohol Dihydrochloride (Win 31,214)**

It was known that Win 31,214 is a compound similar to catecholamines in chemical structure and can be classified as an α-adrenergic stimulant (10). Figure 12 shows the preventive effect of Win 31,214 on the impedance changes induced by histamine. After impedance reduction caused by the first histamine application into a nasal cavity had recovered to control level, Win 31,214 was administered intravenously in a dose of 0.1 or 0.2 mg/kg. 10 min after that, histamine spray was introduced into the other nasal cavity and this time no
Fig. 11. Effect of naphazoline pretreatment on impedance change due to histamine application. Prior to 1% histamine application (H) various concentrations of naphazoline (N) were sprayed. 0.05% naphazoline was apparently effective but not completely. In concentrations higher than that, naphazoline inhibited the histamine effect completely. The time elapse at each interception on the time scale (abscissa) is 10 min.

Fig. 12. Effect of m-amino-\(\alpha\)-(1-aminoethyl)benzyl alcohol hydrochloride (Win 31,214). Effect of 1% histamine spray (H) was inhibited completely by intravenous administrations of Win 31,214 (Win) in doses of more than 0.1 mg/kg. Even in the dose of 0.05 mg/kg, inhibition was almost complete. The time elapse at each interception on the time scale (abscissa) is 10 min.
significant change in impedance was produced. In both cases, arterial blood pressure was not elevated at all. In a similar experiment, 0.05 mg/kg of Win 31,214 was effective to a degree but inhibition was not complete.

Discussion

In the present experiment, histamine spraying into a nasal cavity diminished nasal impedance remarkably in all 35 cases. In this method, no instrument needed to be inserted into the nasal cavity and this may be advantageous in maintaining normal condition of the nasal mucosa. Under such circumstances, no special equipment was necessary to keep airflow normal. Also, the impedance method is much simpler than other methods (5, 12). It requires only insertion of needle electrodes into the nasal wings. Even in such a simple procedure, the responses are reproducible and provide consistent results which may be essential for the evaluation of nasal decongestants. However, in some instances, the tracings of impedance changes were elevated from the control level after recovery from histamine application. This may be due to a drift of the DC signal. However, this does not hinder the evaluation of drugs at all, since, as mentioned before, the test drugs were appraised by the dose required to inhibit histamine effect entirely and the impedance tracings seen in those instances were wholly flat.

Although it was realized that the drift of the baseline may be an unavoidable problem almost inherent in the system when impedance is recorded as a DC signal, in the present experiment DC recording was still adopted, since vascular reaction of the nasal mucosa is induced slowly after histamine application and such a slow change in impedance can be appreciated only in DC tracing. When impedance recording was preliminarily made simply without histamine application in 3 dogs, the tracings were almost flat for 2 h in all instances.

In histological specimens taken from the nasal mucosa when the impedance attained the minimum after histamine spraying, a marked edema in the lamina propria was observed. In those specimens, the stromas were probably soaked in electrolyte-rich surroundings which caused this reduction in electrical impedance. Electron-microscopic examination revealed that histamine acts on the collecting venules and postcapillaries causing the contiguous endothelial cell to separate and for plasma to escape into the stroma (11). Not only edema but also dilatation of the small vessels was conspicuous in those specimens. In order to provoke various symptoms of nasal allergy, it is common in the clinical field to spray a 0.1 % histamine solution onto the nasal mucosa (7). However, when a 0.1 % histamine solution was used as a nasal spray in the dog, uniform responses in impedance changes were rarely evoked in either magnitude or duration. As far as the impedance method is concerned, it seems essential to have a comparable
pattern of impedance change for the evaluation of a nasal decongestant, even though some individual variations are inevitable. Whenever a spray of 1% histamine was used, similar patterns in impedance changes were obtained and such a reproducibility of response after histamine application makes possible the evaluation of nasal decongestants. For this reason, 1% histamine solution was employed as a suitable phlogistic in the present experiment.

In allergy clinics, a 1% acetylcholine solution has also been employed to provoke nasal allergy. However, the incidence of the manifestation of symptoms is much lower than in the case of 0.1% histamine (13). In dogs, acetylcholine was not effective even in 10% solution. From these observations it was concluded that histamine is superior to acetylcholine as a phlogistic regardless of species difference.

Bradykinin is another potent vasodilator and produces edema increasing the permeability of postcapillary venules. Since intense edema can be produced by this agent at concentrations of more than 10^-7% by intracutaneous administration (6), it was anticipated that the spraying of 0.1% bradykinin might diminish nasal impedance appreciably. However, the response after 0.1% bradykinin spray was not impressive and ceased within a few minutes.

It has been reported that when a fixed dose of histamine was given in combination with various doses of an antihistamine, such as diphenhydramine or mepyramine, into the rabbit skin, antihistamine can inhibit the edema formation only when administered in the proper ratio. Other than in such limited ratios, especially when the proportion of antihistamine was large, the edema formation was aggravated (16). In connection with this, it is known that in comparatively higher concentrations, antihistamine alone releases histamine and this action is synergistic with the histamine-releasing effect of basic histamine liberators. In lower concentrations, however, antihistamines inhibited histamine release due to histamine liberators (19). Considering the data presented in these papers, a dose of 0.5 mg/kg of diphenhydramine was chosen and when this was given intravenously, the effect of histamine was obstructed completely, but not with a lesser dose.

In the present experiment, naphazoline was employed as a drug which is representative of drugs being used topically to relieve nasal congestion in acute or allergic rhinitis (21), even though some caution is needed in clinical usage (8). Both 0.1 and 0.5% naphazoline applications abolished histamine effect completely. As mentioned before, in human beings 0.1% histamine solution has been used for the provocation of nasal allergy, and this is one tenth of the histamine concentration employed in the present experiment. On the basis of the concentration difference, it may not be inadmissible to assume that 0.01% naphazoline, i.e., one tenth of the smallest concentration obstructing the histamine effect completely, may be effective in inhibiting nasal symptoms in human beings. At any rate, at least 0.05% naphazoline would probably be enough to
abolish the effect produced by histamine in the human being. The mechanism of the decongestive effect of naphazoline may be based on its vasoconstrictive effect on the nasal mucosa (8).

It has been reported that Win 31,214 is a compound similar to catecholamines in chemical structure and that its action is restricted almost exclusively to the α-adrenergic receptor (10). Because of this property, the compound may constrict the small vessels in the nasal mucosa and counteract edema formation due to histamine. When this compound was given intravenously in a dose of 0.1 mg/kg, it prevented impedance change induced by histamine without any noticeable change in arterial blood pressure. Equivalent dosages in human beings may be sufficient to preclude the symptoms in acute rhinitis which is probably caused by histamine. By comparing the effects of diphenhydramine and Win 31,214, it was noticed that the latter is as potent as the former, or somewhat more efficacious.

References

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