REFLEXES FROM THE UPPER RESPIRATORY PASSAGES IN EXPERIMENTAL INFLUENZA OF FERRETS

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(Received July 1, 1955. Presented by B. S. Preobrazhensky, Member Acad. Med. Sci. USSR)

It was shown in a previous communication [1] that as early as 5-10 minutes after instillation into the nostrils of healthy albino mice of a suspension of infected white mouse tissue containing considerable amounts of Type A influenza virus, strain PR-8, the excitability of the neuroreflex apparatus of the upper respiratory passages rises sharply; this is manifested by enhancement of the inhibitory reflex to the action of a given concentration of carbon dioxide. Instillation of lung tissue suspension from healthy mice causes only insignificant enhancement of neuroreflex excitability, while physiological saline has no effect on the response to carbon dioxide.

In acute experiments on white mice we found that modification of the inhibitory reflex persisted for $1\frac{1}{2}$ - 2 hours after instillation of the virus suspension. It was of interest to investigate the functional state of the neuroreflex apparatus of the upper respiratory passages during the course of development of influenza, since it is known [5,7,8,11,13] that, as a result of the multiplication of the virus in the ciliated epithelial cells, the mucous membrane of the upper respiratory tract, and in particular of the nasal cavities, undergoes considerable morphological change. It might be supposed that, in view of the extensive damage to the mucous membranes, functional changes in the state of the neuroreflex apparatus might be expected. The importance of this problem lies in the circumstance that the upper respiratory passages constitute a major receptor zone, containing the sensory endings of the trigeminal and superior laryngeal nerves, and performing a number of functions essential for the normal vital activities of the organism, including continual and active participation in the neuroreflex regulation of respiration, circulation, and other functions [2,3,9]. It is from this area that such protective-adaptive reflex reactions as sneezing, arrest of inspiration in presence of powerful irritants, tear and mucus production, etc., are effected. Elucidation of changes in the functional state of the neuroreflex apparatus of the upper respiratory passage in influenza may help to solve some of the problems of the pathogenesis of this disease.

The present paper describes the results of an investigation of changes in reflex function of the upper respiratory passage during development of experimental influenza.

The experiments were performed on ferrets, which are irreplaceable for the purpose of obtaining a full clinical picture of influenza, under experimental conditions. As an index of the functional state of the neuroreflex apparatus of the upper respiratory passage, we took the strength of the inhibitory reflex, evoked by inspiration through the nostril, of air of a given carbon dioxide content. The strength of the reflex was derived from the duration of the period of apnea, in seconds.

EXPERIMENTAL METHODS

The experiments were performed on 4 African and 6 forest ferrets, under hexabarbitone narcosis. The animals were given intramuscular injections of 10% hexabarbitone solution, at a rate of 90 mg per kg body weight; sleep supervened in 5-10 minutes, and lasted for $1\frac{1}{2}$ - $2\frac{1}{2}$ hours.

The strength of the inhibitory reflex was determined over a period of 5-7 days before infection, during the course of the disease (daily or every other day), and after recovery (at 2-3 day intervals). Thus 17-18 experiments

were done on each animal. Insufflation of carbon dioxide was performed over periods of 10-20 seconds, and was repeated 5-6 times at 5 minute intervals. Respiratory movements were followed by means of a stethograph.

The animals were infected by intranasal introduction of Type A influenza virus, Strain PR-8, in the form of a 10% suspension of infected mouse lung tissue (amounts of suspension containing several hundred thousand mouse LD_{50} were given). Ferret No. 1 was put in the same cage as ferret No. 2, which was suffering from influenza, and contracted the infection by contact.

The temperature of each animal was measured 3 times daily, rectally. The clinical manifestations of the disease were recorded once for each animal.

Laboratory diagnosis of influenza was performed during the first days of illness, and on the 12th-15th day after infection, by determining the specific influenza antibody titer of the blood serum (by the reaction of inhibition of hemoagglutination).

EXPERIMENTAL RESULTS

Stoppage of breathing occurred as soon as the carbon dioxide acted on the upper respiratory passages, in the phase of expiration, and lasted 4-20 seconds, after which respiratory movements were resumed, and were of greater amplitude (Fig. 1,A). Normal breathing followed a few seconds after cessation of administration of the irritant.

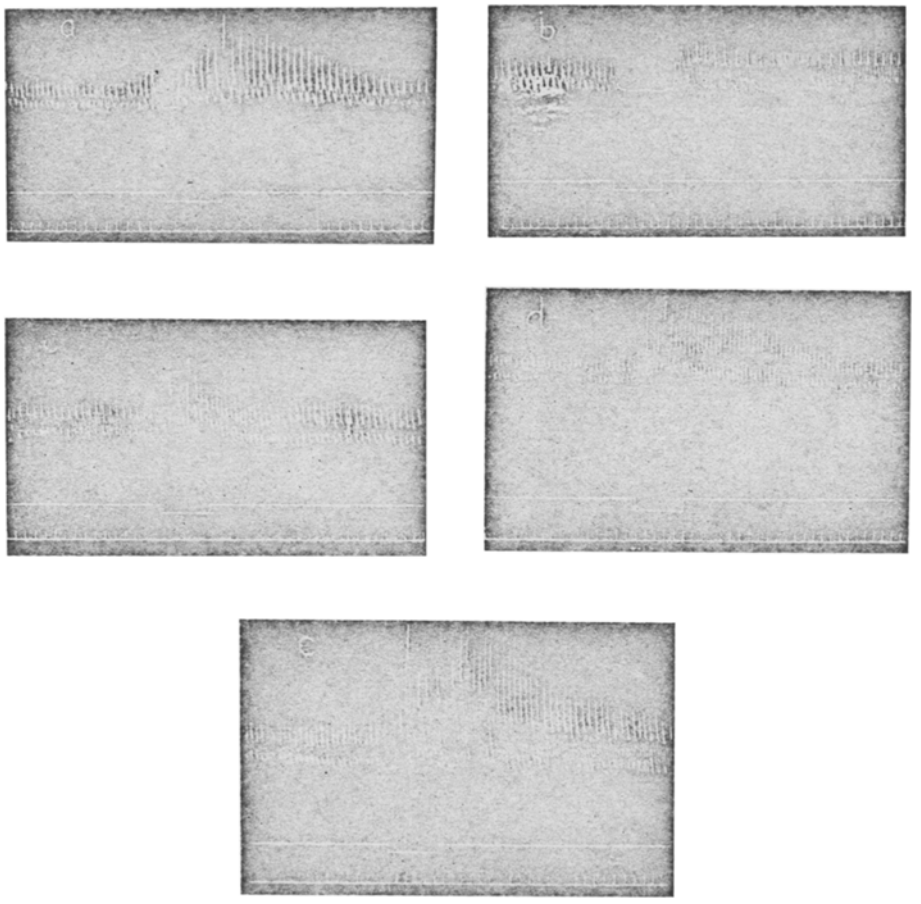

Fig. 1. Arrest of respiration of African ferret No. 2 following irritation of the upper respiratory passages with carbon dioxide, during the course of development of influenza.
A) before infection; B) 10 minutes after instillation of Strain PR-8 virus into the nasal cavity; C) 7 days after infection (animal ill); D) 10 days after infection; E) 18 days after infection (animal clinically recovered).
Explanation of tracings (from above down): pneumogram, signal indicating administration of carbon dioxide, time marker (2 seconds).

The strength of the inhibitory reflex remained at a constant level when measured at 2-day intervals in uninfected animals (Fig. 2, experiments of the 4th, 6th, and 8th of June).

After instillation of influenza virus suspension into the nasal cavity, administration of carbon dioxide, of the same concentration as before resulted in a longer period of respiratory arrest, and the strength of the reflex increased considerably, being doubled for ferrets No. 4 and 9, quadrupled in ferrets No. 2 and 6 (Fig. 1,B), and ten times as great in Nos. 3, 5, and 10. The degree of enhancement varied directly with the dose of virus suspension given.

Instillation of physiological saline did not affect the magnitude of the inhibitory reflex.

Our results afford evidence that the influenza virus acts on the sensory nerve endings of the upper respiratory passages, raising the excitability of the neuroreflex apparatus, and strengthening the inhibitory respiratory reflex.

We thus see that similar results were obtained from experiments on white mice [1] and on African and wood ferrets.

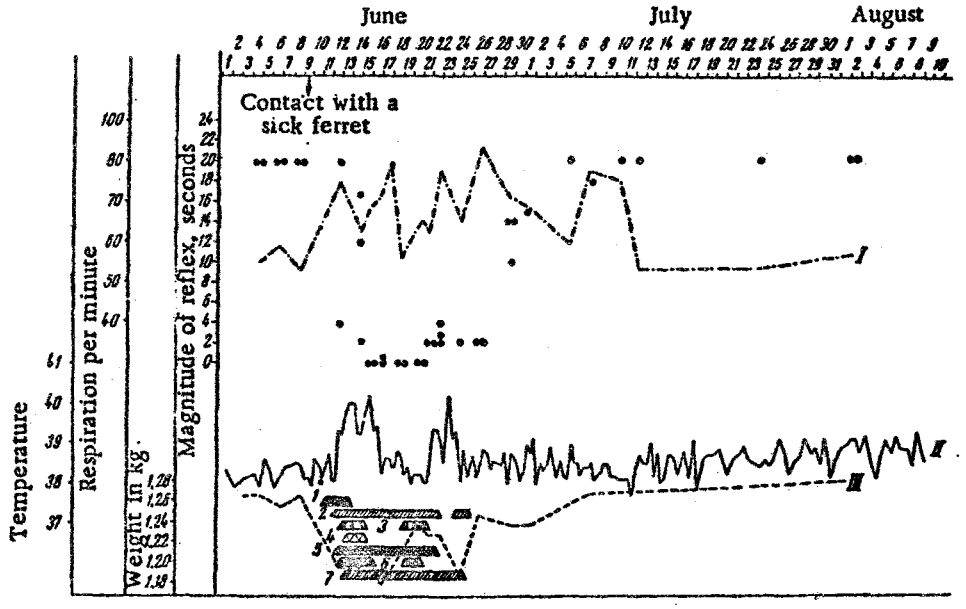

Fig. 2. Results of observation of ferret No. 1.
I) Frequency of respiratory movements; II) temperature; (↓) infection by contact with a sick ferret; (·) duration of respiratory arrest; 1) sneezing; 2) nasal discharge; 3) pallor of tip of snout; 4) conjunctivitis; 5) labored breathing; 6) anoxia; 7) adynamia.

Respiratory arrest due to the action of strong irritants on the upper respiratory passage may be considered as a protective reflex, since it prevents the penetration of harmful substances to the lower respiratory passages [4,12, 14,15]. The greater intensity of this reaction found after introduction of influenza virus is evidence of the enhancement of the defense reaction of the organism. This conclusion is in full agreement with clinical data, and with our observations (greater frequency of the sneeze protective reflex during the first days of illness).

The considerable enhancement of the inhibitory reflex in animals after instillation into the nasal cavity of influenza virus is probably due to the factor stimulating the sensory nerve endings of the nasal mucosa consisting not only of influenza virus particles, with their toxic properties, but also of products of the biochemical processes taking place during the latent period of reaction of the virus with the sensory cells of the nasal mucosa.

The infected animals gave a typical clinical picture of influenza, with manifestations of generalized intoxication and of disease of the nasal cavity.

Temperature rose abruptly on the 2nd day (in some animals on the 3rd or 4th day) after infection. The temperature of healthy animals ranged from 37.9 to 38.8°, and of those suffering from influenza from 40.3 to 41.2°.

In most cases the temperature curve was monophasic, but in two cases it was diphasic. The febrile period lasted 3 days, on the average. The temperature fell sharply in most animals, and lytically in one.

A catarrhal state of the upper respiratory passages developed simultaneously with the rise in temperature. The symptoms appeared on the first day of illness, and were most acute on the 2nd and 3rd days. The nasal discharge was at first of a serous or sero-mucous type, and later became purulent. Formations of crusts around the nostrils were observed in two animals.

The infected animals sneezed repeatedly, especially during the first few days of illness; they were weak, adynamic, drowsy, and anorectic, and their weight fell. At the height of the illness the African ferrets exhibited pallor of the tip of the snout, with signs of conjunctivitis. Breathing was labored, and of increased frequency.

Considerable changes in the functional state of the neuroreflex apparatus of the upper respiratory passage of infected animals were revealed by the action of carbon dioxide. During the incubation period of the disease, as well as, in some animals, during the first days of its course, the excitability of the neuroreflex apparatus was enhanced in some cases, and was normal or depressed in others. At the height of the disease, when the clinical symptoms of influenza are well marked, the inhibitory respiratory reflex is absent (Fig. 1,B and Fig. 2; experiments of the 15th, 16th, and 18th to 21st of June). Respiratory arrest did not follow exposure of the upper respiratory passage to carbon dioxide.

The absence of the reflex was not due to the covering up of the nasal mucosa with inflammatory exudate, which could act as a mechanical hindrance to access of carbon dioxide to the sensory nerve endings. The reflex was in many cases absent in animals with only a slight amount of secretion and, on the other hand, was present in a number of animals with a viscid purulent nasal discharge.

In our opinion, the absence of the reflex is due to functional, and possibly to organic, changes in the sensory nerve endings. The not infrequent incidence of trigeminal neuralgia as a complication of influenza [6,10] lends support to this view.

The depression of the inhibitory reflex reaction to carbon dioxide points to the profound disturbance of the reflex activity of the upper respiratory passages in influenza. It was mentioned above that it is through the exteroception of the nasal mucosa that the organism effects protective-physiological reflexes, and ensures continuous participation of the nose in reflex regulation of respiration and circulation. It may be though that an acute disturbance of regulation of these functions through the upper respiratory passage takes place in influenza, and it is probable that the exclusion of the upper respiratory passage from regulation of respiration is a factor in the pathogenesis of the dyspnea of influenza, uncomplicated by penumonia.

The inhibitory respiratory reflex is gradually recovered after subsidence of the clinical symptoms of influenza, and reaches its initial strength 19-27 days after infection (Fig. 1,D,E; Fig. 2, experiments from June 26 to July 7). Intranasal administration of even very large doses of virus suspension in this period did not affect the magnitude of the inhibitory reflex (Fig. 2), and did not cause disease. The animals were immune; their serum antibody titer varied from 1:8192 to 1:262,144.

The absence of effect of influenza virus on the receptor apparatus of the upper respiratory passage of immune animals supports the view that the condition of immunity to influenza is due not only to elaboration of specific antibodies, but also to modification of the reactivity of the nervous system, and in particular to achievement of a state of non-receptivity of the receptor apparatus of the upper respiratory passage to the action of influenza virus.

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