RESPONSES OF THE CHEMORECEPTORS OF THE CAT CAROTID BODY PERFUSED WITH CELL-FREE SOLUTIONS

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Summary

THE responsiveness of chemosensory discharge recorded from the carotid sinus nerve to chemoreceptor stimulation underwent a progressive attenuation during perfusion of the vascularly isolated carotid body with physiological saline solutions. These attenuations mirrored the reductions of the oxygen usage of the carotid body measured under identical conditions of experimentation. Additions of plasma to the saline perfusates did not prevent the decline of responsiveness during perfusion. Readmission of blood between perfusion periods promptly restored the chemoreceptor responsiveness. It is recommended that extreme caution should be exercised when interpreting results obtained from vascularly isolated carotid bodies perfused with cell-free media.

Introduction

The properties of the chemoreceptors of the carotid body have been extensively studied using vascularly isolated preparations perfused with physiological saline solutions (for reviews see Heymans and Neil, 1958; Anichkov and Belen'kii, 1963; Joels and Neil, 1968). However, Joels and Neil (1968) reported that the use of this preparation not alone greatly reduced the chemosensory responsiveness to chemoreceptor stimuli but could also alter in a qualitative fashion the effects of drugs. These observations, together with the finding that the rate of oxygen utilization by the carotid body falls steeply during cell-free perfusion of the organ (see O'Regan, 1979), question the validity of the use of this type of preparation as a means of studying the properties of the carotid chemoreceptor. The investigation reported here examines the responses of carotid sinus nerve chemosensory preparations to a number of chemoreceptor stimuli during cell-free perfusion and relates these responses to the oxygen usage ($\dot{V}o_2$) of the carotid body measured either in the same experiment or under similar experimental conditions.

Materials and Methods

Pentobarbitone sodium (Nembutal, Abbott; Sagatal, May & Baker Ltd.) was used to induce (i.p. injections, 42-48 $mg.kg^{-1}$) and maintain (i.v. injections, 3-12 mg) anaesthesia in 17 cats (1.7-3.8 kg in weight). Four of the cats were also part of a series used to measure carotid body $\dot{V}o_2$ as reported in a previous paper (O'Regan, 1979). The techniques used to measure systemic arterial blood pressure monitor inflow perfusion pressure, vascularly isolate the carotid body, collect the venous effluent of the glomus and perfuse the organ have been described in that paper.

Recording of chemosensory activity: Chemosensory activities of single or few-fibre preparations were recorded from filaments peeled off the cut carotid sinus nerve using saline wick electrodes. Impulse activity was led to a preampiifier (Grass Model P16) and from thence to an audiomonitor and an oscilloscope (Tektronix 502A) for preliminary examination. The output of the amplifier was also led to a direct-writing ink jet recorder Mingograf 24B; Mingograf 34, Siemens Ltd.) from which permanent

records were obtained. Photographic records were obtained, if required, using a motorized camera (Nihon Kohden) attached to the oscilloscope. Chemosensory activity was recognized by its increase of impulse frequency to perfusion of the carotid body with saline solutions containing sodium cyanide (NaCN, Analar; 20-50 $\mu g.ml^{-1}$) or to stagnant asphyxia of the glomus. Stagnant asphyxia was produced by opening the sidearms of the loops or inflow cannulae to the atmosphere, thereby allowing the pressure of the occluded arterial segment of common and external carotid arteries to fall to atmospheric values (see Fig. 1, O'Regan, 1979). Acetylcholine, (ACh, Sigma; 10-20 $\mu g.ml^{-1}$ perfusate) and 2.4 dinitrophenol (DNP, Hopkins and Williams; 25-50 $\mu g.ml^{-1}$ perfusate) were also used as chemoreceptor stimulants.

Perfusing solutions: Saline solutions (Ringer-Locke and Krebs-Henseleit) or mixtures of these solutions with plasma were used as cell-free perfusates. The composition of the saline solutions and the methods used to obtain the plasma have been described elsewhere (O'Regan, 1979). Additionally, in earlier experiments in this series, plasma was obtained from recently shed ox and human blood or from blood taken from cats experimented upon 1-3 days previously. The ox and human plasma mixtures, when perfused, depressed both chemosensory and barosensory activity recorded from the carotid sinus nerve and their use was discontinued. Stored cat's plasma was also found to be unsuitable as it had variable effects upon chemosensory discharge, possible because of the production of vasoactive and other agents during storage (Phemister and Handy, 1927; Brun, 1949).

Results

Chemosensory responsiveness during perfusion with cell-free solutions: The responsiveness of chemosensory units of the carotid sinus nerve to NaCN, A.U.C., DNP and stagnant asphyxia underwent

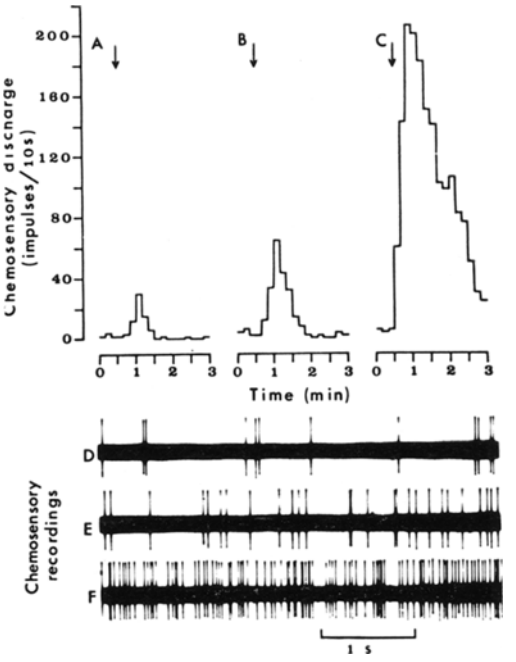

Fig. 1—Responses of a chemosensory unit of the carotid sinus nerve during perfusions of the vascularly isolated carotid body with a Ringer-Locke solution containing NaCN (50 $\mu g.ml^{-1}$). A = cyanide response following 40 min of perfusion with drug-free Ringer-Locke solution; B = cyanide response following 40 min of perfusion with a mixture of drug-free Ringer-Locke solution and plasma (0.5 $ml.ml^{-1}$ perfusate); C = cyanide response following 10 min of blood perfusion carried out subsequent to the response shown in B. D, E and F. are electroneurographic recordings obtained during the peak of the cyanide responses as depicted in graphical forms in A, B and C respectively. Perfusion with the cyanide solution initiated at the arrows. (For further explanation, consult text).

progressive attenuation during cell-free perfusion of the glomus. The altered responsiveness was observed during perfusion with either Ringer-Locke or Krebs-Henseleit solutions and was unaffected by changing the technique of perfusion (see O'Regan, 1979). Additions of plasma to the perfusates (0.1 - 0.6 $ml.ml^{-1}$) did not prevent the decline of responsiveness although the rates of these declines were slowed. Fig. 1 shows the responses of a chemosensory preparation to perfusions of a Ringer-Locke

solution containing NaCN (50 $\mu g.ml^{-1}$), following perfusions of the glomus with either Ringer-Locke solution, a mixture of this solution and plasma or blood. In the upper part of this Fig., chemosensory discharge frequencies during perfusion with cyanide solution (initiated at the arrows) have been plotted against time while the lower part shows electroneurographic recordings of the activity obtained during the peak of the cyanide responses, D, E. and F corresponding to A, B and C respectively. A feeble response to cyanide occurred following 40 min of perfusion with Ringer-Locke solution (Fig. 1 A,D). Following this perfusion with the cyanide solution, blood was admitted to the glomus for 10 min and then, the organ was perfused for a further 40 min with a mixture of plasma and Ringer-Locke solution (0.5 ml $plasma.ml^{-1}$ solution). A somewhat more vigorous response to the cyanide solution occurred after this perfusion (Fig. 1 B,E) than after the Ringer-Locke solution (Fig. 1 A,D) but this response was insignificant compared with that observed after 10 min of blood perfusion (Fig 1 C,F). This response after blood perfusion had a shorter latency, a considerably greater peak frequency and was much more sustained. Exposure of the glomus to blood, even for short periods (2-3 min), always led to a vigorous response to NaCN although a barely perceptible response occurred following a cell-free perfusion carried out previously.

The attenuation of responsiveness during cell-free perfusion mirrored the reductions of glomeral $\dot{V}o_2$ obtained under identical experimental conditions (O'Regan, 1979). In 4 cats, attempts were made to measure glomeral $\dot{V}o_2$ and to record chemosensory discharge from the carotid sinus nerve in the same experiment. In 2 cats, where such procedures were successfully executed, the decline of chemosensory responsiveness to stagnant asphyxia during the first cell-free perfusion followed to a considerable extent the falls of glomeral $\dot{V}o_2$ an example of which can be seen in Fig. 2. Prior to A in this Fig., the carotid body was perfused with blood. At the first arrow, the arterial segment was occluded and the inflow perfusion pressure of this segment reduced to atmospheric values leading to a marked increase of the chemosensory activity of a few-fibre preparation of the carotid sinus nerve (Fig. 2A). The glomus was then perfused with Ringer-Locke solution for 25 min and during this period the $\dot{V}o_2$ of the organ fell by 80% from 0.162 $\mu l.min^{-1}$ to 0.032 $\mu l.min^{-1}$. At this time, the perfusion was discontinued and the inflow pressure reduced to atmospheric values (Fig. 2B), resulting in an asphyxial response which was less than 50% of that obtained prior to the saline perfusion. Saline perfusion was then carried out for a further 30 min and at the end of this period the glomeral $\dot{V}o_2$ was 0.014 $\mu l.min^{-1}$, less than 10% of its initial value. The asphyxial response (Fig. 2C) was now less than 20% of the value prior

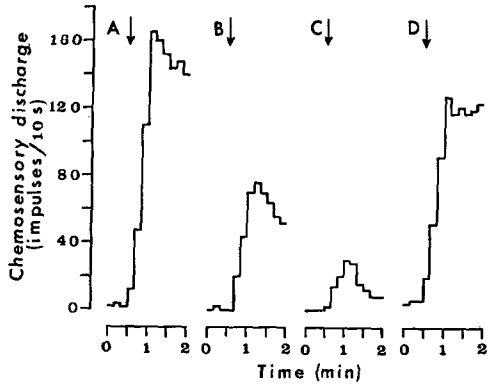

Fig. 2—Responses of a few-fibre chemosensory preparation of the carotid sinus nerve to stagnant asphyxia of the carotid body. A=asphyxial response following perfusion of the glomus with blood; B=asphyxial response following a 25 min perfusion of the glomus with Ringer-Locke solution; C=asphyxial response following a further 25 min of perfusion of the glomus with Ringer-Locke solution carried out subsequent to the response shown in B; D = asphyxial response following an 8 min perfusion of the glomus with blood carried out subsequent to the response shown in C. Stagnant asphyxia initiated at the arrows. (For further explanation, consult text).

to the saline perfusion. Between C and D blood was readmitted to the glomus for 8 min, and at the end of this period of perfusion an asphyxial response was obtained which was approximately 75% of the initial value (Fig. 2D). Thus as glomeral $\dot{V}o_2$ fell so did chemosensory responsiveness to asphyxia and there was a considerable recovery of this responsiveness after readmission of blood.

Restoration of chemosensitivity after prolonged perfusion with solutions containing chemoreceptor stimulants: Saline solutions containing cyanide perfused through the vascularly isolated carotid body initially cause marked enhancements of chemosensory activities recorded from the carotid sinus nerve but these enhancements are not sustained, the discharge frequencies falling to low values within a few minutes of perfusion (Joels and Neil, 1962, 1968; Anichkov and Belen'kii, 1963). In 8 cats, carotid bodies were perfused with cell-free solutions containing either NaCN, ACh or DNP until such time as insensitivity to the chemoreceptor stimuli developed. Attempts were then made to restore the responsiveness to the stimuli by perfusing the glomus with various drug-free media. All chemoreceptor stimuli caused "exhaustion" of the chemosensory mechanism within 6 min of perfusion. Subsequent to this "exhaustive" process, cell-free media were perfused through the glomus but these perfusates, even those with plasma added, were unable to restore the chemosensitivity. On the other hand, readmission of blood to the carotid body, even for periods as short as 2 min, invariable restored the responsiveness to the chemoreceptor stimuli. A typical sequence of events observed in these experiments can be seen in Fig. 3. Perfusion of the glomus with a Ringer-Locke solution containing NaCN (40 μg.ml^{-1}), initiated at the first, fifth and ninth arrows, caused a marked increase of the discharge of a few-fibre chemosensory preparation of the carotid sinus

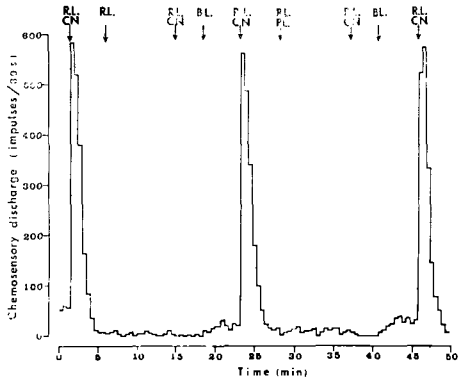

Fig. 3—Restoration of chemosensitivity following prolonged perfusion of the vascularly isolated carotid body with Ringer-Locke solution containing NaCN. Chemosensory discharge recorded from a few-fibre preparation of the carotid sinus nerve. Sequence of perfusions indicated by the arrows. BL=onset of perfusions with blood; RL =onset of perfusion with Ringer-Locke solution; R.L.CN = onset of perfusion with Ringer-Locke solution containing NaCN (40 μg.ml^{-1}); R.L.PL= onset of perfusion with a mixture of Ringer-Locke solution and plasma (0.45 ml plasma.ml^{-1} solution). Prior to the first cyanide perfusion the glomus was perfused with blood. Note that blood perfusion always restored chemosensitivity while cell-free media were ineffective in this respect.

nerve following blood perfusion. On the other hand, cell-free perfusion of the "exhausted" glomus failed to restore the responsiveness to the cyanide solution (initiated at the third and seventh arrows).

Prolonged perfusion of a solution containing a chemoreceptor stimulant usually led to insensitivity not alone to that agent itself but also to other stimulants as well. In the example shown in Fig. 4, perfusion with a saline solution containing ACh (10 μg.ml^{-1}) failed to excite chemosensory discharge after "exhaustion" of the receptor by a prolonged cyanide perfusion. Responsiveness to ACh was restored after readmitting blood. After insensitivity to ACh had been produced by prolonged perfusion with this stimulant, cyanide was now ineffective. Readmission of blood once more restored the responsiveness to cyanide. However, in 20% of chemosensory preparations studied, a response,

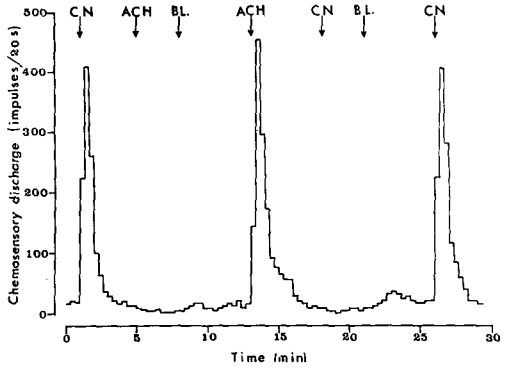

Fig. 4 — Responses of the chemosensory discharge of a few-fibre preparation of the carotid sinus nerve following prolonged perfusions of the vascularly isolated carotid body with Ringer-Locke solutions containing the chemoreceptor stimulants NaCN and ACh. Sequence of perfusions indicated by the arrows. ACH = onset of perfusions with a solution containing ACh (10 $\mu g.ml^{-1}$); BL = onset of perfusion with blood; CN = onset of perfusions with a Ringer-Locke solution containing NaCN (50 $\mu g.ml^{-1}$). Note that insensitivity to ACh followed prolonged perfusion with the cyanide solution and vice versa.

albeit considerably reduced, to solutions containing ACh could still be elicited after prolonged perfusion of the glomus with cyanide. The opposite effect of a response to NaCN following prolonged perfusion with solutions containing ACh was also noted.

Discussion

This investigation confirms the observation of Joels and Neil (1968) and Whalen and Nair (1977) that the responsiveness of chemosensory discharge recorded from the carotid sinus nerve becomes markedly attenuated during perfusion of the vascularly isolated carotid body with saline solutions. It further shows that the longer the period of saline perfusion the greater the reduction of chemosensitivity, and that these reductions mirror to a considerable extent the declines of carotid body $\dot{V}o_2$ occurring under similar experimental conditions (O'Regan, 1979). Exposure of the glomus to blood between saline perfusion periods enhances chemosensitivity as it does glomeral $\dot{V}o_2$.

The causation of the marked decrease in chemosensitivity during saline perfusion is unknown. Factors which could have been implicated in the decreases of glomeral $\dot{V}o_2$ during saline perfusions have been discussed in a previous paper (O'Regan, 1979) and such factors may also be relevant as causative elements in the reductions of chemosensitivity. However, none of these factors can satisfactorily explain the behaviour of the carotid body during saline perfusion. In particular, the suggestion of Joels and Neil (1968) that a factor present in blood, presumably in the plasma, is necessary to maintain the normal responsiveness of the chemoreceptors is not supported in the present investigation. Plasma additions to the saline solutions, although they did slow the rate of the reductions in chemosensitivity, did not prevent such reductions during perfusion. Furthermore cell-free perfusates, with or without plasma added, were incapable under the conditions of these experiments of restoring the responsiveness of the chemoreceptors in circumstances in which chemosensitivity had been lost consequent upon prolonged perfusion of the glomus with solutions containing chemoreceptor stimuli.

It is possible that plasma itself, rather than the plasma-saline mixtures, may have been more effective but its use in the present investigation was not feasible. The small quantities of plasma obtainable from the same cats whose carotid bodies were used for perfusion purposes necessitated a diluted plasma mixture. Plasma solutions obtained from oxen, humans and other cats were unsatisfactory as perfusion media. At any rate, the viscosity of plasma so increased the resistance of passage of the fluid through the narrow tubes of the perfusion systems as to preclude its use in an undiluted form.

Fay (1970) asserted that the use of mixtures of plasma and saline solutions aided in maintaining the chemosensitive responsiveness of his carotid body pre-

parations. He observed a "normal" response of chemosensory activity recorded from the whole carotid sinus nerve to NaCN after the carotid body had been perfused with plasma-saline mixtures for over an hr. Recording from the the whole sinus nerve is unreliable as the number of active chemosensory units being recorded is highly variable depending on the recording conditions, associated nerve damage and the electronic noise levels so the term "normal" as used by Fay has no significance whatsoever. In the present investigation single or few-fibre chemosensory units were used and, although a response to cyanide could be elicited an hr after the beginning of cell-free perfusion, such a response was but a feeble reflection of a "normal" response. It is known that the peak discharge rate to NaCN of chemoreceptor A fibres can be as high as 40 impulses.s^{-1} (Fidone and Sato, 1969).

The postulate of Joels and Neil (1968) that a precursor present in blood might be required for the anabolism of a transmitter substance in the glomeral cells cannot be completely eliminated by the results of the present investigation. It may be that the substance in question is labile and that it was destroyed during the processes used in the present investigation to obtain the plasma and form the cell-free solutions. In this context, it is known that there is a rapid turnover of the catecholamines of the carotid body (Grönblad and Korkala, 1977), and it is possible that the rate of synthesis of these agents may be profoundly affected during saline perfusion of the glomus owing to lack of a precursor substance. Catecholamines, especially dopamine, may be of considerable importance in the chemoreceptive process within the carotid body (Mitchell and McDonald, 1975; Llados and Zapata, 1978). Furthermore, choline has been shown to prolong the life and responsiveness of *in vitro* carotid body preparations acting either as a precursor for glomeral ACh (Eyzaguirre and Zapata, 1968) or as a substrate in oxidative metabolism (Joels and Neil, 1968).

Joels and Neil (1962) and Krylov and Anichkov (1968) demonstrated that chemosensory units could still respond to ACh when their chemosensitivity to cyanide had been lost following prolonged perfusion of the glomus with this agent. In the present investigation, 80% of units were inexcitable to ACh under the same circumstances and those which did respond did so in a much attenuated fashion. A possible explanation for the variation in these results may be the type of fibre used for recording purposes and the ease of accessibility of exogenously applied ACh to the zone of excitation of the nerve ending (Paintal, 1967; Fidone and Sato, 1969).

An important point which arises is whether the vascularly isolated carotid body perfused with saline solutions is a valid preparation for the study of chemoreceptors. It is of little moment if chemoreceptor responses are at least qualitatively correct, if not quantitatively so, and fortunately, this does seem to pertain for most stimuli. However, the effects of drugs may be so modified using this preparation as to produce results qualitatively different from blood perfused carotid bodies. A good example of a modified action of a drug which has caused much difficulty in interpretation is given by hexamethonium. Joels and Neil (1962), using the vascularly isolated preparation perfused with saline, were unable to repeat the observations of Douglas (1952) that hexamethonium, a nicotinic cholinoceptor antagonist, abolished the responses of chemosensory activity to exogenously applied ACh leaving excitation by natural stimuli unaffected. Joels and Neil's evidence argued in favour of a cholinergic mechanism of excitation within the carotid body during chemoreceptor excitation. Subsequently, Joels and Neil (1968) found that additions of blood to the saline perfusates gave

results with hexamethonium similar to those of Douglas, who had in his experiments used a blood perfused carotid body. Thus, the presence of blood in the perfusing solutions can fundamentally affect the action of a drug.

Eyzaguirre and his coworkers have extensively studied the carotid body chemoreceptors using an *in vitro* preparation (Eyzaguirre and Lewin, 1961; Eyzaguirre and Koyano, 1965a; Eyzaguirre and Zapata, 1968). In this preparation, the carotid body, removed from the cat, is placed in a stream of Locke's solution with the carotid sinus nerve lifted into overlying oil for the purpose of recording chemosensory potentials. On the surface, this preparation should be unreliable as a means of studying the chemoreceptor properties of the glomus. Yet, chemosensory discharge can be recorded for prolonged periods using this preparation without apparent loss of responsiveness to chemoreceptor stimulants. Indeed, ACh not alone engenders a chemosensory response after prolonged superfusion with cyanide solutions but even restores the cyanide sensitivity (Ezyaguirre and Koyano, 1965b). Peak discharge rates of single or few-fibre preparations of the carotid sinus nerve to chemoreceptor stimulation are high and, presumably, these fibres innervate superficially located, well-oxygenated areas of the glomus. Paradoxically, it appears that the chemoreceptor responses of *in vitro* carotid body preparations are similar to blood perfused organs and superior to glomi perfused with saline solutions. However, there are several disquieting features concerning the effects of drugs when this *in vitro* preparation is employed. Thus, mecamylamine, a cholinoceptor antagonist, abolished or greatly reduced the chemosensory responses to both exogenously applied ACh and to natural stimuli when the *in vitro* carotid body preparation was used (Eyzaguirre and Zapata, 1968). These workers reasoned that because this blocking agent penetrates membranes more readily than other antagonists such as hexamethonium, it could more easily reach the locus of excitation of the nerve ending normally excited by endogenously produced ACh. However, Sampson (1971) using an *in vivo* blood perfused organ found responses to natural stimuli and NaCN after administering doses of mecamylamine that blocked responses to exogenously applied ACh. While Nishi and Eyzaguirre (1971) argued that the dose of mecamylamine used by Sampson was inadequate nonetheless, a recent refined pharmacological analysis by McQueen (1977) confirmed the findings of Sampson and he further showed that in his blood perfused preparations the effects of other drugs such as atropine and hemicholinium differed from those of Eyzaguirre and Zapata in important respects.

It is well known that intracarotid injections of dopamine can repeatedly depress the chemosensory discharge recorded from blood perfused carotid bodies (Black *et al*, 1972; Sampson, 1972; Ilados and Zapata, 1978). On the other hand, Zapata *et al* (1969) found dopamine to be ineffective using *in vitro* carotid body preparations, an observation in line with the findings of Joels and Neil (1968) who employed a saline perfused carotid body. Subsequently, Zapata (1975) did find that chemosensory discharge could be depressed when using the *in vitro* carotid body preparation but a rapid desensitization to the amine occurred so that on repeating the dopamine applications there was either no effect of the agent or excitation was elicited. Furthermore, desensitization to dopamine occurred after applying other catecholamines to the *in vitro* glomus. The *in vitro* preparation had so modified the action of dopamine as to fundamentally alter the effects which can be constantly observed when the carotid body is blood perfused.

In conclusion, the vascularly isolated carotid body perfused with saline solutions and also, probably, the *in vitro*

superperfused preparation have a number of deficiencies compared with blood perfused glomi. These preparations would seem to be too unreliable for the study of chemoreceptor properties and the results obtained in these circumstances should be interpreted with extreme caution.

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