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Coma induced by Cholinergic Activation of a Restricted Region in the Pontine Reticular Formation

—A Model of Reversible Forms of Coma—

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Abstract

We previously demonstrated evidence suggesting increased neural activity in the pontine reticular formation ventromedial to the principal nucleus of locus coeruleus after experimental concussive head injury in the cat. We report here that microinjections of small doses of the cholinergic agonist carbachol (0.4 μg in 0.2 μl, bilaterally) into this restricted brain stem region of the awake cat produces a reversible, flaccid, comatose state analogous to that of the lowest score on the Glasgow Coma Scale, the grading system commonly employed in assessments of human coma. Microinjection of the local anesthetic tetracaine into the same region failed to produce detectable effects, and therefore the carbachol effects were probably due to activation of pathways within the injection sites that actively inhibit various aspects of the animals' responsiveness to the external environment. The flaccid, comatose state produced by carbachol was not the result of epileptic processes, since neither epileptic electroencephalographic discharges nor convulsive movements were observed. The muscarinic antagonist atropine reversed the carbachol effects. We shall also discuss possible implications of these data in terms of mechanisms of flaccid coma associated with good recovery in humans.

Key words: central cholinergic system, coma, concussion, flaccidity, pontine inhibitory area, locus coeruleus

Introduction

Since the essential feature of coma is reduced behavioral responsiveness to external stimuli and inner need,[20,25,34,75] a comatose state is best evaluated by measuring observable responses to external stimuli. In humans, a coma associated with complete absence of somatic motor responses, including spinally or bulbospinally mediated reflexes, together with loss of postural muscle tone (hereafter referred to as a flaccid state) is assigned the lowest score on the Glasgow Coma Scale (GCS).[75] Since the demonstration by Lindsley *et al.*[50,51] that acute mesencephalic interruption of the ascending reticular activating system (RAS)[54,55,60] could produce a comatose state, coma has generally been attributed to interruption of an ascending flow of mesencephalic reticular impulses.[1,21-24,28,64,65] However, flaccid coma cannot be produced solely by experimental lesions of the RAS, since spinally and bulbospinally mediated reflexive responses are preserved in the presence of such lesions.[31,65,73,76,83] On the other hand, it has been shown that brain stem lesions involving the pontomedullary reticular formation can produce flaccidity.[27,65,76,83]

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Thus, flaccid coma, such as that associated with the lowest score on the GCS, is generally considered to indicate broad brain stem damage and to have a poor prognosis[10,15] (also see ref. 13). However, flaccid coma associated with a good recovery is not extremely rare.[15,56] Nevertheless, no rigorous attempt to understand the mechanisms of reversible coma has been made.

The most representative example of reversible flaccid coma is that which occurs as a consequence of concussive head injury.[15,19,49,64,78,82] Typically, motor responses and muscle tone are absent, and therefore the state has been referred to as paralytic coma.[19,64,82] Furthermore, these symptoms can resolve without any overt cellular or vascular damage.[19] Recently, Hayes *et al.*[29] reported evidence suggesting increased neural activity in a restricted brain stem region within the pontine reticular formation ventromedial to the principal nucleus of locus coeruleus after experimental concussive head injury in the cat. This area includes a region recently shown to be populated with cholinoceptive, cholinoceptive-cholinergic, and cholinergic cells.[46] Other data, from both head injured humans[77,84] and experimental animals,[12,70] suggest that activation of central cholinergic mechanisms may be responsible for the production of certain clinical signs of concussive head injury, such as stupor and loss of muscle tone.[12]

These observations prompted us to examine, in normal, awake animals, the behavioral consequences of experimental activation of an area in which increased neural activity was postulated to occur after concussive head injury. Our results demonstrate that a flaccid, comatose state can be produced by purely neurally mediated processes (*i.e.*, activation of a certain neural population in the brain stem). This finding raises the possibility that flaccid coma in a certain subset of comatose patients may not necessarily represent a pathological depression of broad brain stem areas, including the pontomedullary reticular formation.

Materials and Methods

I. Surgical preparation

Thirty adult cats (2 to 4 kg) were used in this study. All cats were surgically prepared under general anesthesia (sodium pentobarbital 30 mg/kg, i.v.) and aseptic conditions. Two 26-gauge, permanent guide tubes (length, 30 mm, manufactured by Small Parts, Inc., Miami, Florida) were implanted symmetrically through the cerebellum and permanently secured to the skull with dental acrylic and stainless steel screws. All guide tubes were implanted at an angle of 30° or 45° to the coronal plane in order to avoid the bony tentorium and to allow studies of various regions of the rostral pontine tegmentum. The guide tubes were plugged with stylets (length, 30 mm) when not in use. Stainless steel screws were affixed to the skull in the parietal and occipital regions for recording electroencephalograms (EEGs). Flexible wires were implanted in nuchal muscle for recording electromyograms (EMGs).

II. Microinjection procedures

One week after surgery, the cats were habituated to the experimental situation. They were microinjected while restrained in loose-fitting canvas bags (manufactured by Haver Lockhart Laboratories, Kansas City, Kansas) from which their heads projected. Injection cannulae were constructed from 33-gauge steel tubes (outer diameter, 200 μm; inner diameter, 100 μm) and connected to a small piece of 26-gauge steel tube so that a predetermined length (31.0 to 34.5 mm) of 33-gauge steel tubing protruded from the 28-gauge steel tubes. The other ends of the 26-gauge steel tubes were connected to lengths of polyethylene tubing (PE 20, manufactured by Becton, Dickinson and Co., Parsippany, New Jersey), which were fitted to the needles of microsyringes (manufactured by Hamilton Co., Whittier, California). The cholinergic agonist carbachol (Sigma) and other solutions were microinjected bilaterally and sequentially through the left and right cannulae. In an effort to minimize the mass of neural tissue affected by the microinjections, smaller unilateral doses and volumes were used (0.4 μg in 0.2 μl, 0.9% saline vehicle adjusted to pH 7.4) than had been employed in any previous studies using microinjection techniques in the cat. The drug was infused at a constant rate of 0.2 μl/min. The injection cannulae were left in place for an additional 2 minutes to minimize back diffusion of drugs when the cannulae were withdrawn. Successive microinjections, each separated by more than 4 days, were made along the cannula tracks in 1.0 mm increments up to 4.5 mm beyond the tips of the guide tubes. For pharmacological studies, the muscarinic antagonist atropine sulfate (Sigma, 1.2 μg in 0.6 μl) or the nicotinic antagonist mecamylamine hydrochloride (Sigma, 0.4 μg in 0.6 μl) was also microinjected at the same sites into which carbachol (0.6 μg) had been microinjected 8 minutes earlier. In addition, we studied the effects of systemically administrated atropine (0.5 to 1.0 mg/kg, i.v., n=3) on the behavioral effects produced by carbachol (0.6 μg) microinjected 10 minutes earlier. We also examined the behavioral effects of tetracaine

hydrochloride (Sigma, 0.5 μg in 0.5 μl) or the saline vehicle alone (0.2 to 2.0 μl) microinjected at the same sites at which carbachol produced maximal behavioral effects.

III. Coma Scale for the cat

Following widely applied criteria for human coma,[20,25,75] we designed a scale to grade coma in the cat. As detailed below, the deepest coma was defined as a state in which the postural muscles (m. score) and eyes (e. score) were totally unresponsive to intense external stimuli, including lifting and vigorous shaking, tapping, toe-pinching, pin-pricking, and suspension by limbs. Manipulations that result in tissue damage were not used.

m. Score: Responsiveness of postural muscles was scored as the best momentary response to maximal external stimuli, according to the following scale: 4, possessing normal locomotor and running abilities; 3, noticeably weak yet still capable of supporting body weight; 2, incapable of supporting body weight yet still able to support the head; 1, incapable of supporting the head yet still possessing spinally mediated reflexes; 0, completely unresponsive.

e. Score: Eye-opening responses consisted of palpebral widening and retraction of nictitating membranes in response to external stimuli. Scores were graded as follows: 4, full retraction of palpebrae and nictitating membranes throughout 1-minute periods in the absence of external stimulation; 3, relaxation of palpebrae and nictitating membranes but responsiveness to light touch and stroking of the hair of the flank; 2, relaxation but responsiveness to firm tapping of the flank; 1, relaxation but responsiveness to maximal external stimuli; 0, relaxation and unresponsiveness to even maximal external stimuli. Since contraction of musculus orbicularis oculi is necessary for full closure of the eyes in cats, those in a comatose state or under general anesthesia do not typically show complete eye closure. Thus, the eye-opening response of the palpebrae was defined as widening of the narrowed palpebral fissure.

These scoring criteria are comparable to the motor and eye-opening components of the GCS. Since animals with scores of m.0/e.0 never vocalize, this combination of scores is equivalent to the deepest coma, graded as the lowest GCS score, in humans. Such a state is reliably produced by experimental concussive head injury in the cat.

IV. Physiological recordings

Respiration and heart rates, parietal and occipital EEGs, and nuchal EMGs were monitored in cats whose behavior was simultaneously studied. Signals were amplified by a Grass P511J amplifier (manufactured by Grass Instruments Co., Quincy, Massachusetts) and recorded on a Gould Brush 480 polygraph (manufactured by Gould, Inc., Cleveland, Ohio).

V. Histological confirmation

At the end of the experiment, the animals were anesthetized with pentobarbital, microinjected with India ink (0.1 μl) through the cannulae at predetermined depths and then transcardially perfused with a buffered formalin (10%) solution. Serial frozen coronal sections (40 μm) were cut and stained for subsequent histological confirmation of cannula placement by an experimenter uninformed of the behavioral effects of the carbachol microinjections. Only those observations obtained from paired injection sites showing less than 0.3 mm asymmetry, as confirmed histologically, were included in this analysis. We employed the nomenclature described by Berman.[11]

VI. Statistical analyses

Student's (unpaired and paired) *t*-tests were employed for parametric analyses. Variability is always expressed as the standard error of the mean. Nonparametric analysis was performed by the chi-square test, unless stated otherwise.[16]

Results

Among 64 bilateral injection sites in the rostral pontine tegmentum, we found that microinjection of carbachol produced scores of m.0/e.0 in three sites, m.0/e.1 in three sites, and m.1/e.0 in one site, with latencies ranging from 3 to 9 minutes following injection. These sites were clearly clustered in a restricted region in the pontine reticular formation ventromedial to the principal nucleus of locus coeruleus (Fig. 1). No abnormal behavioral changes, such as excitation or convulsive movements of muscles, were seen. The difference between the scores of sites within this area and sites out of this area was significant ($p < 0.001$).

During states scored as m.0, animals consistently and completely lost resting tone in postural muscles of the jaws, neck, trunk, and limbs (Fig. 2). Nuchal EMGs showed neither spontaneous activity nor responses to maximal external stimuli (Fig. 3). There was no preferential effect on flexor or extensor muscles, so that the animals hung slackly when lifted. As m.0 scoring criteria indicate, no responses requiring the use of postural muscles were elicited even when maximal external stimuli were applied. Even spinally mediated reflexive movements, such as

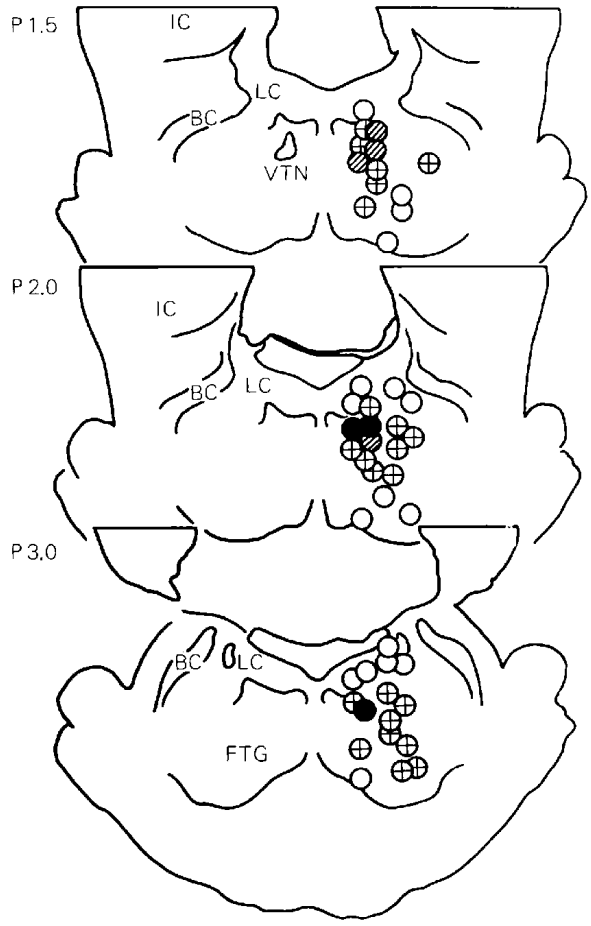

Fig. 1 Histological mapping of injection sites and their associated m. and e. scores in three coronal levels between 1.5 and 3.0 mm posterior to the interaural line. Solid circles indicate m.0/e.0 scores; hatched circles, m.0/e.1 or m.1/e.0 scores; circles with cross, other combinations of scores lower than m.3 or e.3; open circles, m.3/e.3 or higher scores. See text for the definitions of scores. IC: inferior colliculus, LC: principal nucleus of locus coeruleus, BC: brachium conjunctivum, VTN: ventral tegmental nucleus of Gudden, FTG: gigantocellular tegmental field.

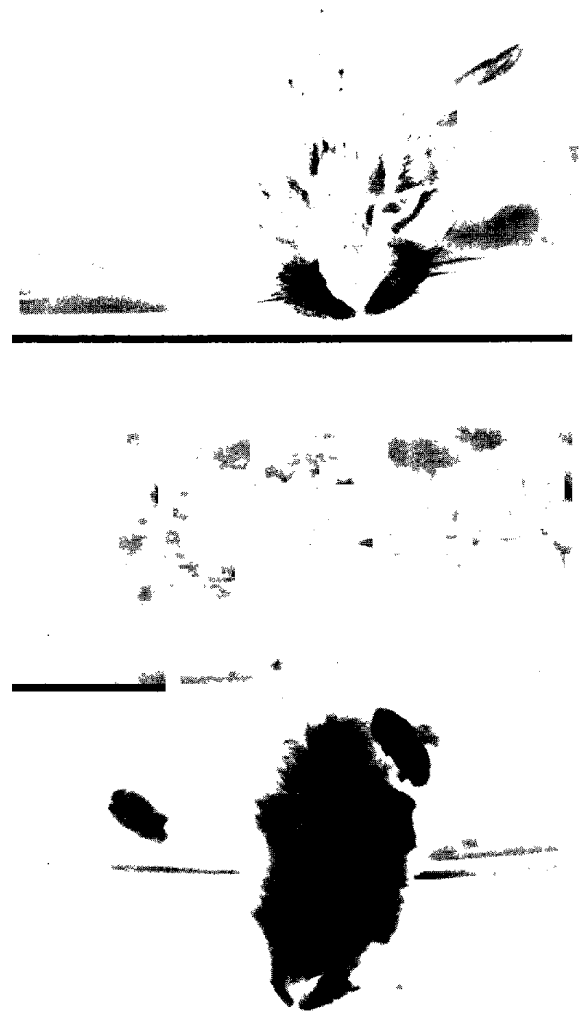

Fig. 2 Photograph showing behavioral consequences 5 minutes after carbachol microinjection into the site associated with m.0/e.0 scores. Note that postural muscles are flaccid, palpebral fissures are narrowed, and nictitating membranes are relaxed. The palpebrae of comatose cats (such as concussed or anesthetized animals) are usually not completely closed.

stretch or flexion reflexes, were not observed. However, somatic motor activity associated with the blink reflex was always preserved even in animals that were scored e.0.

As the scoring criteria indicate, even maximal external stimuli elicited no retraction of the relaxed nictitating membranes and narrowed palpebrae during a state scored as e.0 (Fig. 2). Pupils were constricted (<1.0 mm), and maximal external stimuli failed to elicit pupillary responses. The photic reflex was also not convincingly elicited in sites associated with a score of e.0. Maximal external stimuli failed to elicit changes in respiration and heart rates in two of the sites associated with a score of e.0. During a state scored as m.0/e.0, the EEG was characterized by continuous low-voltage desynchronization. No epileptic discharges or abnormal slow waves were observed. It was usually difficult, on EEG, to clearly observe any further increase in frequency or reduction in amplitude in response to maximal external stimuli (Fig. 3). Scores of m.0/e.0 lasted less than 10

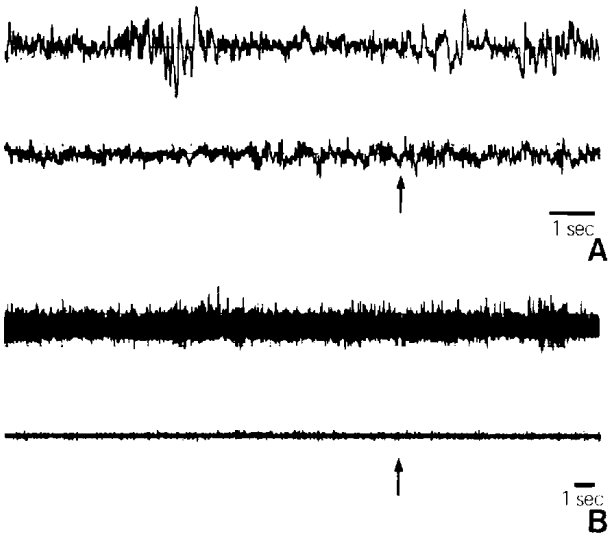

Fig. 3 Examples of parietal EEG (A) and nuchal EMG (B) before (*upper traces*) and 5 minutes after (*lower traces*) carbachol microinjections into the site associated with m.0/e.0 scores. Arrows indicate pinching the toe pads (2.0 kg/sq mm). Note desynchronization of EEG and abolition of spontaneous EMG activity after carbachol microinjection. Pinching failed to produce noticeable changes in the EEG and EMG.

minutes and thereafter both scores gradually increased.

Irregular eye movements resembling rapid eye movement (REM) were observed following carbachol microinjection into a medial area of the pontine tegmentum, including the medial aspect of gigantocellular tegmental field (in left 1.0 to 2.0). The occurrence of REM-like eye movements, however, was not associated with the occurrence of low m. and/or e. scores, either with respect to time course or to injection sites that produced these carbachol effects. In sites associated with low m. and/or e. scores within the dorsomedial pontine tegmentum, low m. and e. scores were reliably observed earlier than REM-like eye movements, if such eye movements occurred at all. In contrast, REM-like eye movements were elicited most clearly in an area ventral to the dorsomedial pontine tegmentum, in which relatively higher m. and e. scores were usually observed.

The effects of microinjection of the muscarinic antagonist atropine were examined in four of the sites associated with low m. and e. scores. Atropine microinjected 8 minutes after carbachol microinjection significantly accelerated the recovery of both scores, according to records obtained 20 minutes after carbachol microinjection (Wilcoxon signed rank test, $p<0.05$). Intravenous administration of atropine more clearly antagonized the carbachol effects, within 1 minute in all three sites examined. Animals scored as m.0/e.0 began to move nearly normally within a few minutes after systemic atropine administration. In contrast, microinjection of mecamylamine into four sites associated with low m. and e. scores showed no significant antagonism of any carbachol effects. Microinjection of the local anesthetic tetracaine ($n=4$) or saline vehicle alone ($n=4$) into sites associated with m.0/e.0 scores following carbachol microinjection produced no detectable behavioral effects.

Discussion

This study demonstrated that a flaccid, comatose state resembling that usually graded as the most severe form of coma can be produced by microinjection of small doses of cholinergic agonist into a restricted brain stem region. This finding is surprising in view of the rather widely held belief that such pharmacological agents as general anesthetics must affect broad areas of the brain in order to produce a state similar to coma.[17]

Since no obvious behavioral effect was produced by microinjection of the local anesthetic tetracaine[66] into the sites at which flaccid coma had previously been elicited by carbachol microinjection, the flaccid coma produced by carbachol appears to be a result of activation of pathways within the injection sites. As inferred from published data on microinjection of various dyes,[61] the extent of diffusion of carbachol, when maximal carbachol effects were observed, was estimated to be less than 1.0 mm. Thus, only cholinoceptive cell populations within a restricted brain stem region appear to be activated by the procedure employed in this study. The antagonism of carbachol effects by atropine but not by mecamylamine indicates that muscarinic rather than nicotinic receptors mediate the activation of these pathways. Furthermore, the effects of carbachol microinjection were not due to epileptic processes, since neither epileptic discharges on EEGs nor convulsive movements were observed. These observations strongly suggest that the flaccid, comatose state produced by carbachol was the result of physiologically and neurally mediated processes activated within a restricted brain stem region.

Earlier studies indicated that activation of muscarinic cholinergic mechanisms in the pontomesencephalic tegmentum can produce suppression of behavioral responsiveness to external

stimuli.[9,26,32] However, no effort was previously made to localize the region associated with the most pronounced unresponsiveness to external stimuli, nor did earlier work systematically relate behavioral changes produced by microinjection of cholinergic agonists to the behavioral characteristics of coma. In our study, the region producing a state similar to flaccid coma is located dorsal to an area from which paradoxical sleep-like behavior has been reported[2] and medial to an area from which pure postural atonia has been reported[59,81] after carbachol microinjection. Systematic exploration of the effects of carbachol microinjected into various sites of the pontomesencephalic tegmentum recently carried out in this laboratory[37,44] indicated that a cholinoceptive pontine inhibitory area (CPIA) within the dorsal pontine tegmentum regulates a number of different functions supporting responsiveness to external stimuli independently of other manifestations of paradoxical sleep.

One of the most salient features of the CPIA is its involvement in descending control of spinal motor output and/or sensory input.[37,44] The region associated with production of a state similar to flaccid coma in this study corresponds to the medial part of the CPIA, activation of which can maximally suppress postural somatomotor and sympathetic visceromotor output from the spinal cord.[37] Thus, the flaccidity observed in animals scored as m.0 is attributable to descending influences on somatomotor output from the spinal cord. Interestingly, this effect appears to be restricted to postural muscles, since the blink reflex was consistently preserved.

Abolition of eye-opening responses to external stimuli, scored as e.0, is also partly attributable to descending influences on sympathetic visceromotor output, since retraction of nictitating membranes is mediated exclusively by sympathetic activities.[7,14,68,69] Constricted pupils and relaxed palpebrae may also be at least partially attributable to reduced resting sympathetic tone. However, palpebral opening and pupillary widening in normal cats, when elicited as components of orienting reactions to novel external stimuli, may be mainly produced by nonpostural striated muscles and inhibition of parasympathetic tone,[48,80] respectively. Thus, there may be other functional changes produced by carbachol that could suppress these orienting reactions to external stimuli (see ref. 40).

The desynchronized EEGs following carbachol microinjection suggest that suppression of orienting reactions may not be induced by generalized depression of forebrain activities, such as that produced by disruption of the RAS, since lesions in the RAS are associated with increased slow wave EEG activity.[50,51] Rather, it appears that interactions between the central nervous system and the external environment are suppressed relatively independently of the functioning of other intrinsic brain activities. Such a possibility is in accord with the observation that the CPIA appears to regulate various aspects of interactions between higher brain processes and the external environment.[37,40,44,45]

Since coma is a behaviorally defined state in which observable responses are reduced, the criteria for coma do not necessarily imply generalized depression of brain activity. For example, a comatose state associated with a desynchronized EEG has been reported following caudal mesencephalic lesions in humans.[35,52,53] In such cases, neural mechanisms that support responsiveness to external stimuli are disrupted by lesions, while intrinsic brain activities supported by the RAS seem to remain intact. During the flaccid, comatose state that we investigated in this study, a similar condition appeared to be induced by purely neurally mediated processes.

This observation raises the possibility that activation of the medial part of the CPIA may underlie some forms of clinically observed flaccid coma. As mentioned earlier, studies of regional rates of glucose utilization indicate that activity within this area indeed increases after concussive head injury in the cat.[29,30,42] In addition, the behavioral consequences of concussive head injury include a reversible, flaccid, comatose state that resembles the carbachol effects described here.[30,42] A comatose state following concussive head injury in the cat can occur in the absence of slow waves on the EEG. Moreover, transections of the brain stem at different levels have indicated that the rostral pontine reticular formation, including regions associated with production of a flaccid, comatose state in our study, is necessary to produce electrophysiological indices of flaccidity of postural muscles following concussive head injury. Thus, it appears plausible that activation of this region may be responsible for at least some components of flaccid coma observed following concussive head injury.

Further studies are required to determine whether or not there is a specific pathological state that triggers the activation of this region of the CPIA. It is possible that certain patterns of functional derangement within other brain regions result in a predominance of activity within this neural population. Such a consideration implies that this same neural population may also contribute to flaccid coma of other etiologies. On the other hand, this region is located near an area that has been reported

to produce changes in forebrain blood flow[18,43,67] and cerebrovascular permeability[67] following cholinergic or electrical stimulation. Although more detailed hypotheses obviously await further research, it is possible that mechanisms underlying some form of flaccid coma are at the same time responsible for certain changes in the intracranial environment.[39] In this regard, it is interesting to note that a certain group of patients who exhibited flaccid coma and had a good outcome reportedly had high intracranial pressure.[56]

Except for cases in which lesions of the pontomedullary reticular formation are implicated, the mechanisms of clinically observed flaccid coma are not fully understood. Among patients with severe head injury treated in our clinical head injury research center,[10] 8% exhibited flaccidity on admission and 76% of these flaccid patients died.[15] Despite this high mortality rate, there is apparently a group of flaccid patients who make a good recovery.[15,49,56] Thus, although flaccid coma is indeed associated with extreme unresponsiveness, such a state does not always indicate the most severe insult to the brain. It is possible that a reversible pathophysiological state in certain brain regions may underlie reversible flaccid coma. Regarding this possibility, it is important to recognize that features of flaccid coma may result not only from depression of the RAS and descending pontomedullary facilitatory systems but also from a predominance of activity within endogenous neural systems, such as those within the CPIA,[37,44] which can inhibit responsiveness even at the level of the spinal cord. More than 3 decades ago, Araki and his colleagues[8,33,62,85] demonstrated several lines of evidence indicating that abnormal excitation of neural activities within the mesencephalic or the pontomesencephalic region induced either by mechanical, electrical, or chemical stimulation results in coma. They reported their experiments under the term "coma-puncture."[3-6] Stockard *et al.*[74] recently suggested, based on their experiences with a large series of post-traumatic coma, that in some cases coma is a more active process than was formerly supposed, and involves overaction of inhibitory centers or some other imbalance in the brain stem activating system. To the best of our knowledge, Araki and his colleagues were the first to demonstrate experimental evidence indicating that excitation rather than destruction of certain neural populations within the brain stem can produce coma. It should be mentioned that there are remarkable similarities in the anatomical, neurochemical, behavioral, and neurophysiological features of coma observed by Araki and his colleagues[57,58,63,71,72] and those reported in this paper.[36-38,40,42] There are, however, several points of disagreement regarding the anatomical and neurochemical aspects of the neural processes that underlie observed coma; these have been described in detail elsewhere.[79] We believe that we have confirmed the presence of an active neural process, originally suggested by Araki and his colleagues, in the production of certain forms of coma and, furthermore, clarified the anatomical and neurochemical features of one neural population involved in this process.

Conclusion

Our study has demonstrated that a flaccid, comatose state analogous to human coma associated with the lowest score on the Glasgow Coma Scale can be reversibly produced in the cat by cholinergic activation of a restricted region in the pontine reticular formation ventromedial to the principal nucleus of locus coeruleus. This flaccid, comatose state appeared to result from active suppression of mechanisms responsible for various aspects of responsiveness. Flaccidity of postural muscles may be one manifestation of the neural processes that mediate such suppression. Several lines of evidence suggest that enhanced activity within this region may provide at least one neural basis of flaccid coma following concussive head injury.

Rather than the nonspecific depression of broad areas of the brain stem previously invoked to explain clinically observed flaccid coma, it may be that an imbalance of brain stem functions, such as that produced experimentally in our study, is responsible for certain cases of reversible, flaccid coma of diverse etiologies.

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