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Effects of sodium pentobarbital anesthesia and neurotrophic factor on the maintenance of acetylcholinesterase and butyrylcholinesterase in the preganglionically denervated superior cervical ganglion of the cat

(sympathetic nervous system)

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ABSTRACT **In continuation of a previously reported study, the superior cervical ganglia of cats were preganglionically denervated bilaterally under sodium pentobarbital anesthesia. The following day cats were reanesthetized and infused via the common carotid artery with an aqueous extract of cat brain, spinal cord, and sciatic nerves for periods of 24, 12, 6, and 3 hr, without ligation of the external carotid or lingual arteries as was done previously. Values for acetylcholinesterase and butyrylcholinesterase of superior cervical ganglia at 48 hr postdenervation were all considerably above those of denervated controls. However, values for cats infused with 0.9% NaCl solution and for noninfused cats in which sodium pentobarbital anesthesia was maintained during the 24- to 48-hr postdenervation period were similarly elevated, to approximately twice the values in denervated controls. Ligation of the external carotid and lingual arteries at 24 hr postdenervation was found to oppose the preservation of acetylcholinesterase and butyrylcholinesterase contents of the ganglia induced by barbiturate anesthesia. When arterially ligated cats were infused with extract for periods of 12, 6, or 3 hr, beginning 24 hr postdenervation, acetylcholinesterase contents of superior cervical ganglia were elevated significantly above those of reanesthetized, arterially ligated controls after 12 and 6 hr but not after 3 hr of infusion, at 48 hr postdenervation.**

We have recently reported that the intracarotid infusion in cats of a crude aqueous extract (Ext) prepared from cat brain, spinal cord, and sciatic nerves, from 24 to 48 hr after preganglionic denervation of the superior cervical ganglia (SCG), results in a marked decrease in the loss of ganglionic acetylcholinesterase (acetylcholine acetylhydrolase; EC 3.1.1.7; AcChoEase) and butyrylcholinesterase (acylcholine acylhydrolase; EC 3.1.1.8; BtChoEase) that otherwise is found at 48 hr postdenervation (1). The incorporation of aprotinin (Trasylol) in the extracts resulted in a loss of effectiveness, although the differences between values in cats infused with this preparation and the denervated controls were still highly significant. Cats infused only with aprotinin showed a relatively small but significant preservation of AcChoEase but not of BtChoEase. The probable reasons for these findings were discussed.

Immediately prior to infusion, the external carotid (EC) and lingual (L) arteries were ligated bilaterally to divert the major portion of the infused Ext to the SCG. A series of denervated cats, in which the same arterial ligations were performed without infusion, showed slightly but significantly higher ($P < 0.05$) values for AcChoEase but not for BtChoEase than the denervated controls.

The present study was undertaken to simplify the assay procedure prior to attempts to isolate and identify the neurotrophic factor in the Ext. Two modifications were tested: (*i*) omission of the arterial ligations and (*ii*) shortening of the original 24-hr infusion period. Unexpected results were obtained, which have led to the demonstration that continual anesthesia with sodium pentobarbital also causes a significant reduction in the loss of AcChoEase and BtChoEase in denervated ganglia. Additional complications regarding interpretation of the earlier results have been resolved, with confirmation of the neurotrophic activity of the Ext. Clinical implications regarding the use of barbiturate anesthesia in the early treatment of brain and spinal cord injury are discussed.

METHODS

Preparation of the infusion Ext, anesthetic and surgical procedures, and the methods for homogenization of ganglia, for determination of their AcChoEase, BtChoEase, and protein contents, and for calculation of statistical significance of mean differences were identical with those reported previously (1), with the exceptions noted below. Under sodium pentobarbital anesthesia (35 mg/kg, intraperitoneally) ≈1 cm was resected from both cervical sympathetic trunks; the wound was sutured and Combiotic (penicillin with dihydrostreptomycin, Pfizer, 0.5 ml, intramuscularly) was given. The following day cats were again anesthetized as before, and infusion of the Ext was begun ≈24 hr after denervation. In the present initial series, ligation of the EC and L was not performed. Cats were given artificial respiration through a tracheal catheter attached to a Palmer pump, and a continuous intravenous infusion of 5% glucose/0.45% NaCl, to a total of ≈250 ml; heparin, 50 units/kg, intravenously, was given every 6 hr during infusion. Deep anesthesia was maintained by repeated intravenous doses of sodium pentobarbital. With shortening of the infusion period, the rate of infusion was progressively increased to deliver the same approximate volume of Ext in each experiment. The infusion was terminated at 24, 12, 6, or 3 hr; in all cases except the first, operative wounds were then sutured. Anesthesia was repeated and SCG were excised exactly 48 hr after denervation.

As a result of initial findings, two series were expanded from the previous study: (*i*) denervation controls (cats anesthetized

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Abbreviations: AcChoEase, acetylcholinesterase; BtChoEase, butyrylcholinesterase; EC, external carotid artery; Ext, nerve tissue extract; L, lingual artery; SCG, superior cervical ganglion.

and denervated bilaterally, allowed to recover, and reanesthetized and SCG taken 48 or 72 hr after denervation); (*ii*) arterial ligation controls (denervated bilaterally; the following day, reanesthetized and EC and L ligated bilaterally, reanesthetized and SCG excised 48 hr postdenervation). An additional series consisted of cats that were denervated bilaterally and then maintained under continual sodium pentobarbital anesthesia until the time of sacrifice and removal of SCG, 48 or 72 hr postdenervation. Finally, cats were infused with Ext after denervation and subsequent bilateral arterial ligation as in the previous study, but for shorter periods (12, 6, and 3 hr).

RESULTS

Findings from the previous study (1) that are directly applicable to the present investigation are summarized in Table 1.

Infusion of Ext in denervated, nonarterially ligated cats, starting 24 hr postdenervation, for periods of 24, 12, 6, and 3 hr (Table 2) resulted in substantially higher values for ganglionic AcChoEase and BtChoEase at 48 hr postdenervation than in denervated controls (Table 1, group 2, 48 hr) in all cats, with the possible exception of the 6-hr infused cat (Table 2, cat O). However, there was no apparent correlation between the period of infusion and AcChoEase and BtChoEase contents of the SCG. As an additional control, a cat was then infused for 6 hr with 0.9% NaCl solution under otherwise identical conditions. Surprisingly, the AcChoEase and BtChoEase contents of the SCG (Table 3, cat T) were approximately the same as those of the unligated cats that had been infused with Ext for 3–24 hr. When this experiment was repeated with omission of heparin, results were similar (Table 3, cat U). The major factor that differed between these two experiments and the denervated controls appeared to be the administration of sodium pentobarbital anesthesia on the day after denervation. Accordingly, two cats were denervated and reanesthetized with sodium pentobarbital throughout the following day and prior to sacrifice at 48 hr; one cat (V) also received the usual doses of heparin. The AcChoEase and BtChoEase contents of their SCG (Table 3, cats V and Y) were similar to those of the reanesthetized, saline-perfused cats. The pooled mean values for the continually anesthetized saline infused and noninfused cats (Table 3, cats T–V and Y) were AcChoEase, 250 ± 23, and BtChoEase, 444 ± 41 nmol of substrate hydrolyzed per mg of protein per min; the value for AcChoEase was nearly twice that of the denervated controls of the earlier series. To provide a more accurate means of comparison, the latter group was expanded (Table 4, item 1a). The difference between the mean values for AcChoEase contents of SCG in continually anesthetized and nonanesthetized cats sacrificed 48 hr postdenervation was highly significant ($P < 0.001$); that for BtChoEase was not ($P < 0.1$).

Four cats were sacrificed at 72 hr postdenervation, during which time they were given repeated intraperitoneal doses of sodium pentobarbital and daily infusions of ≈300 ml of 5% glucose/0.45% NaCl, subcutaneously. Although values for gan-

Table 1. Summary of results from previous study (1) applicable to present investigation

Group	Procedure	Substrate hydrolyzed, nmol/mg of protein per min: AcChoEase	BtChoEase
1	Normal cat SCG	449 ± 42 (8)	590 ± 61 (8)
2	Preganglionically denervated SCG		
	48 hr postdenervation	129 ± 6 (6)	401 ± 18 (6)
	72 hr postdenervation	86 ± 6 (2)	389 ± 5 (2)
3	Preganglionically denervated SCG; EC and L ligated 24 hr and SCG excised 48 hr postdenervation	158 ± 10 (6)	444 ± 28 (6)
4	SCG denervated; cats arterially ligated and infused with Ext 24–48 hr postdenervation; SCG excised at 48 hr	354 ± 35 (6)	602 ± 65 (6)

Numbers of SCG in parentheses. Mean ± SEM = $[\Sigma d^2/n(n-1)]^{1/2}$; where $n = 2$, SEM is equivalent to the range.

Table 2. Effects of infusion of nervous system Ext for various periods on the AcChoEase and BtChoEase contents of the SCG of the cat, 48 hr after preganglionic denervation*

Cat	SCG	Infusion time, hr: Post-denervation	Total	Volume infused, ml	Substrate hydrolyzed, nmol/mg of protein per min: AcChoEase	BtChoEase
G	R	24–48	24	188	259	391
G	L				321	468
N	R	24–36	12	141	257	410
N	L				304	490
O	R	25–31½	6½	194	177	301
O	L				173	331
S	R	23–26	3	110	265	436
S	L				270	423

R, right; L, left.
*EC and L not ligated.

Table 3. Effects of continual anesthesia with sodium pentobarbital on the AcChoEase and BtChoEase contents of the SCG of the cat after preganglionic denervation*

Cat	SCG	Time of sacrifice, hr post-denervation	Sodium pentobarbital (cumulative dose), mg/kg†	Substrate hydrolyzed, nmol/mg of protein per min: AcChoEase	BtChoEase‡
T§	R	48	78	246	494
	L			198	364
U§	R	48	101	236	377
	L			213	362
V	R	48	97	194	340
	L			220	381
Y	R	48	97	319	588
	L			375	646
Mean ± SEM (T, U, V, Y)				250 ± 23¶	444 ± 41
B	R	72	117	60	261
	L			74	329
C‖	R	72	93	67	287
E	R	72	172	126	156
	L			125	117
F	R	72	196	78	27
	L			71	16
Mean ± SEM (B, C, E, F)				86 ± 10	292 ± 20

R, right; L, left.
*EC and L arteries not ligated.
†Exclusive of dose (≈50 mg/kg, intravenously or intraperitoneally) given immediately prior to sacrifice.
‡Values for cats E and F omitted from calculations of mean. See *Discussion*.
§Cats T and U infused via the R common carotid artery with 130 and 193 ml, respectively, of 0.9% NaCl over 6 hr.
¶More than 48-hr denervated control ganglia (Table 4, group 1, 48 hr), $P < 0.001$.
‖Left SCG incompletely denervated (absence of Horner's sign) and therefore discarded.

Table 4. Data from previous study (1) expanded for present investigation

Group	Procedure	Substrate hydrolyzed, nmol/mg of protein per min: AcChoEase	BtChoEase
1	Preganglionically denervated SCG		
	48 hr postdenervation	122 ± 5 (10)	349 ± 27 (10)
	72 hr postdenervation	82 ± 8 (6)	339 ± 30 (4)
2	Preganglionically denervated SCG; cats reanesthetized and EC and L ligated 24 hr and SCG excised 48 hr postdenervation	167 ± 10* (8)	412 ± 29 (8)

Number of SCG in parentheses.
* Less than SCG from 48-hr denervated, continually anesthetized cats (Table 3), $P < 0.005$; more than denervated SCG (group 1), 48 hr, $P < 0.001$.

glionic AcChoEase were somewhat higher in the two cats (Table 3, cats E and F) that received extremely high doses of sodium pentobarbital than in the two (cats B and C) that were given moderate doses, the differences for the aggregate values were not significantly higher than those of the controls (Table 4, group 1, 72 hr).

In the previous study, it was concluded that anesthetization and ligation of the EC and L on the day after denervation produced a small increase in the AcChoEase and BtChoEase contents of the SCG at 48 hr (Table 1, groups 2, 48 hr, and 3). In view of the immediately foregoing findings regarding the effects of anesthesia, it is evident that (*i*) the anesthetized, arterially ligated group must serve as the controls against which the effects of anesthetization, arterial ligation, and infusion of Ext are compared, and (*ii*) the effects of arterial ligation must be compared against reanesthetized cats. Therefore, the arterially ligated group was also expanded (Table 4, group 2). When results with 24-hr Ext-infused cats of the previous series (Table 1, group 4) were compared with those of the arterially ligated cats (Table 4, group 2), the differences were still significant ($P < 0.001$ for AcChoEase and $P < 0.05$ for BtChoEase), thus confirming the neurotrophic effect of the extract. Comparison of previous results of infusion with aprotinin-containing extract and with aprotinin alone against the arterially ligated series showed that the differences were also significant for AcChoEase ($P < 0.025$ and $P < 0.001$, respectively), but not for BtChoEase.

When the SCG of the reanesthetized, arterially ligated cats were compared against those of the cats anesthetized on the day after denervation (Table 3), it was found that ligation produced a significant decrease ($P < 0.005$) in the content of AcChoEase rather than an increase as had been concluded previously.

A series of arterially ligated cats was then infused with Ext for periods of 12, 6, and 3 hr, beginning ≈24 hr postdenervation. At 48 hr postdenervation, the AcChoEase contents of the SCG were elevated significantly above that of the arterially ligated controls in the 12- and 6-hr infused groups; in the 3-hr group, values were approximately the same as those of the controls (Table 5). In none of these groups did BtChoEase contents differ significantly from the controls.

Table 5. Effects of infusion of nervous system Ext for 12, 6, or 3 hr on the AcChoEase and BtChoEase contents of the SCG of the cat, 48 hr after preganglionic denervation*

Cat	SCG	Infusion time, hr: Post-denervation	Total	Volume infused, ml	Substrate hydrolyzed, nmol/mg of protein per min: AcChoEase	BtChoEase†
H	R	25–37	12	141	357	70
	L				315	26
N	R	24–36	12	166	170	301
	L				186	313
O	R	26–38	12	240	261	293
	L				281	324
Mean ± SEM (H, L, M)					262 ± 30‡	308 ± 7
X	R	26½–33	6½	119	256	506
	L				295	621
I	R	26–32	6	184	293	352
	L				236	440
J	R	26–32	6	201	207	274
	L				236	293
Mean ± SEM (X, I, J)					254 ± 14§	414 ± 55
Q	R	26–29	3	175	183	260
	L				175	255
S	R	26–29	3	165	145	217
	L				176	247
Mean ± SEM (Q, S)					170 ± 8	245 ± 10

R, right; L, left.
* EC and L arteries ligated.
† Values for cat H omitted from calculations of mean. See *Discussion*.
‡ More than 48-hr denervated, EC and L arterially ligated cats (Table 4), $P < 0.005$.
§ More than 48-hr denervated, EC and L arterially ligated cats (Table 4), $P < 0.001$.

DISCUSSION

The present findings have advanced the primary aim of the ongoing investigation, the identification of the neurotrophic factor in crude extracts of the nervous system, by demonstrating (*i*) the necessity of ligating the EC and L to obtain maximal effects in the Ext infusion experiments, (*ii*) the appropriate controls (cats anesthetized and arterially ligated the day after denervation) against which results of infusion with Ext should be compared, and (*iii*) that the period of infusion can be shortened to 6 hr for assays of neurotrophic activity.

Perhaps the most important finding is that continual anesthesia with sodium pentobarbital results in a marked decrease in the loss of AcChoEase and BtChoEase contents of SCG during the 48-hr period after preganglionic denervation. Although the mechanism of this action remains to be determined, in all probability it reflects a diminution or retardation of nerve cell damage. This could be investigated by electron microscopic histochemistry (2) and could be examined further by studies of the effects of barbiturate anesthesia on other enzymes and systems of preganglionically denervated ganglia.

Barbiturates have been shown to suppress energy metabolism and presumably thereby provide protection for the brain against hypoxia (3) and arterial hypotension (4). A few years ago it was reported that treatment of head injury patients with anesthetic doses of sodium pentobarbital for 72 hr or longer, in addition to other measures, resulted in marked improvement in survival rate and extent of ultimate recovery (5). Beneficial effects were attributed solely to reduction of elevated intracranial pressure. Because this was unlikely to be a factor in the present study, it is suggested that sodium pentobarbital might be of value in the early treatment of brain and spinal cord injury uncomplicated by increased pressure.

The basis for the enhanced loss of AcChoEase and BtChoEase contents of the SCG in cats in which the EC and L were ligated in comparison with the unligated cats that received pentobarbital anesthesia on the day after denervation is unexplained. It can be speculated that the ligations increased blood flow to the SCG in the former group and that this opposed the beneficial suppression of metabolic activity by the barbiturate.

No explanation can be offered for the inordinately low assay values for BtChoEase obtained in all ganglia of cats of several series (Table 3, cats E and F; Table 4, cat G; Table 5, cat H) during one week (May 9–13, 1983) of the present study or for the relatively low values obtained thereafter. The method was checked with freshly prepared reagents; repetition of assays on these homogenates and on frozen samples of earlier homogenates gave approximately the same values as originally. It is possible that this reflects in part a seasonal variation in the BtChoEase contents of SCG noted previously (W. A. Koelle, personal communication). Values for these cats are recorded but are omitted from calculations of means. The values for AcChoEase in these ganglia were all consistent with those of other ganglia in the same series.

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