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Effect of Anesthetics on the Interaction between Pressor and Depressor Reflexes in Dogs (40941)[1]

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Abstract. The interaction between the pressor reflex to aortic nerve (AN) stimulation and the depressor reflex to carotid sinus nerve (CSN) stimulation was studied in the perfused hind limbs of dogs using different anesthetic agents commonly used in physiological studies. With chloralose, pentobarbital, or a combination of urethane and chloralose, stimulation of the depressor reflexes augmented the vasoconstrictor effects of the pressor reflex. Animals anesthetized with urethane alone did not demonstrate this interaction between these reflexes. The results suggest that chloralose and pentobarbital enhance the aortic nerve pressor reflexes rather than suppress the carotid sinus nerve depressor reflexes.

The interaction between two reflex systems may occur in a simple additive manner or in a manner which involves more complex interactions between central neurons. We have previously reported (1) that pressor reflexes to stimulation of the aortic nerve (AN) are augmented during stimulation of depressor afferents in the carotid sinus nerve (CSN). In these experiments a situation existed where an excitatory influence operated upon a background of low vasomotor activity due to the inhibition caused by CSN stimulation. Physiologically, in disturbances such as hypoxemia, baroreceptor reflexes would operate upon an elevated background of vasomotor activity. One aspect of the present studies was to determine if the interaction between these reflex systems depended upon the initial level of vasomotor activity.

A second aspect of these studies was to determine how some of the anesthetics commonly used in physiological studies affect the interaction between these reflexes. Early studies by Douglas *et al.* (2) demonstrated that the cardiovascular response to simultaneous stimulation of pressor and depressor afferents in the CSN of the cat was dependent on the anesthetic used. Chloralose and pentobarbital reversed the depressor effects of CSN stimulation into pressor effects in decerebrate and urethane-anesthetized cats. These investigators concluded that this reversal was due to selective inhibition of the depressor reflexes, leaving the pressor reflexes essentially unaltered. The present studies suggest that chloralose and pentobarbital may augment the pressor reflexes through more complex actions than suppressing the activity of the depressor reflexes.

Methods. The experiments were conducted on a total of 31 dogs (17–27 kg). These animals are divided into four experimental groups based on the anesthetic used.

Group 1. Ten dogs were anesthetized with α-chloralose (100 mg/kg, iv) 30 min following premedication with morphine (3 mg/kg sc). The initial anesthetic was supplemented by iv drip at the rate of about 25 mg/hr.

Group 2. Eight animals were anesthetized with urethane (750 mg/kg, ip) after premedication with morphine. No supplemental anesthetic was given during the course of the experiment.

Group 3. Nine animals were anesthetized with a combination of urethane (500 mg/kg) and chloralose (50 mg/kg) intravenously. No additional anesthetic was given in these experiments. This combination has been used in other studies (3, 4) which suggested that chemoreceptor stimulation resulted in cutaneous vasodilation. In one of these animals one hind limb was skinned from the groin to the paw. Circulation to the paw was excluded by tight ligatures. The other hind limb was left in-

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tact. Both limbs were perfused independently. The responses in the skinned and intact limbs to AN stimulation were compared to determine whether changes in flow distribution between skin and muscle influenced our results. The data from this experiment are not included in the tables.

Group 4. Five animals were anesthetized with sodium pentobarbital (30 mg/kg, iv). The initial dose was supplemented as necessary to maintain a surgical level of anesthesia.

In several of the animals in the above groups 5 μg of norepinephrine was given intra-arterially into the limbs to test the effect of the anesthetic agents on the sensitivity of the vascular smooth muscle to adrenergic stimuli.

The trachea was intubated and all the animals were artificially ventilated with room air. Ventilatory rate was adjusted to keep the gaseous composition and the pH of the arterial blood in the normal range. Both vagi were cut in the midcervical region to eliminate cardiopulmonary reflexes.

Both CSN were carefully isolated from their junction with the glossopharyngeal nerve back to their origin in the carotid sinus. These nerves were cut close to the sinuses and drawn into silver ring electrodes. Both AN were dissected free from the vagosympathetic trunks and identified in a manner previously described (5, 6). The AN were cut and their central ends drawn into silver ring electrodes.

The hind limbs were perfused at a constant rate of flow by a finger pump. The lower abdominal aorta was dissected free and all aortic branches except the common iliac arteries were ligated. A centrally directed cannula was tied into the isolated aortic segment to provide pump inflow. A second cannula, directed peripherally, was tied into the distal aorta just above the bifurcation to provide perfusion of the hind limbs. An electromagnetic flow sensor incorporated in the pump circuit monitored pump output. Limb perfusion pressure was measured from a branch of the femoral artery with a Statham pressure transducer. Heparin (3 mg/kg) was given initially and supplemented hourly (0.5 mg/kg). Systemic pressure was measured from the superior thyroid branch of the carotid artery. Pressures and flows were recorded on a Beckman recorder.

The AN and CSN were stimulated with rectangular stimuli from a dual-channel stimulator (Grass S-88). The CSN was stimulated with 100-msec trains of stimuli (2 trains/sec, 2–20 stimuli per train). The duration of individual stimuli was 0.1 msec. Stimulus voltage (1–4 V) was adjusted to cause a near maximum depressor response in the perfused limbs during intermittent stimulation with 2 trains/sec, 20 stimuli/train. This level of stimulation did not alter breathing in the spontaneously breathing animal.

The AN was stimulated with one stimulus synchronized to the beginning of each CSN stimulus train. The duration of the AN stimuli was 1.0 msec and the voltage varied (0.8–3 V) so that pressure in the perfused limbs rose to 20 to 30 mm Hg from a base level of 100 mm Hg. The animals anesthetized with urethane required the higher stimulus in this range and it was sometimes necessary to use two stimuli/train in order to obtain pressor responses of this size. Spontaneous breathing was usually increased in rate and depth by AN stimulation.

In a typical protocol hind limb flow was first adjusted so that limb perfusion pressure was about 100 mm Hg. The AN was stimulated and the pressor response was recorded. The CSN was then stimulated with 2 trains/sec, 20 stimuli/train. Limb flow was increased to maintain limb pressure at 100 mm Hg and the ipsilateral AN was again stimulated. After obtaining these data hind limb flow was adjusted so that perfusion pressure was about 180 mm Hg. The CSN depressor curves to progressive increments in the number of stimuli per train from 2 to 20 were obtained. Following recovery from this series, the AN on the same side was stimulated, limb flow was adjusted until pressure was about 180 mm Hg, and the CSN stimulation series was repeated.

The data within experimental groups were compared by the standard paired *t*

test; comparisons between groups were made by the unpaired t test (7). All data are presented as means ± SD.

Results. Table I compares the pressor responses to AN stimulation in the perfused limbs before and during stimulation of CSN depressor reflexes. In all animals, except those anesthetized with morphine–urethane, the AN pressor response was enhanced ($P < 0.01$) during CSN stimulation. Figure 1 illustrates the difference in the AN pressor response before and during CSN stimulation in the dogs anesthetized with urethane (750 mg/kg) alone and in those anesthetized with a combination of urethane (500 mg/kg) and chloralose (50 mg/kg). These experiments were selected for presentation because the initial values of limb vascular resistance (LVR) both before and during CSN stimulation are similar. On the average control LVR was higher in the animals anesthetized with urethane–chloralose. In this latter group the pressor response to AN stimulation is greater during CSN stimulation than before. These responses are also typical of those seen in the dogs anesthetized with morphine–chloralose or with sodium pentobarbital. Little or no augmentation of the pressor response was observed in the animals anesthetized with morphine–urethane. The values of initial LVR were lower ($P < 0.05$) in the animals anesthetized with urethane. The level of LVR during CSN stimulation did not differ ($P > 0.05$) among the experimental groups.

These observations suggest that either chloralose and pentobarbital act on some component in the reflex pathway to enhance the interaction between these reflexes or that urethane suppresses this interaction. In one dog, anesthetized with morphine–chloralose, an anesthetic dose of urethane (750 mg/kg) was given. The control AN pressor and the CSN depressor responses were essentially unchanged by the addition of urethane. CSN stimulation enhanced the AN pressor response to about the same degree after the addition of urethane as before in this dog.

The control pressor responses to equivalent AN stimulation were generally

TABLE I. PRESSOR RESPONSES IN THE PERFUSED LIMBS TO STIMULATION OF THE AN BEFORE AND DURING CSN STIMULATION AND TO NOREPINEPHRINE (ia)

N/T[a]	Limb perfusion pressure (mm Hg ± SD)	Initial LVR (mm Hg·liter^{-1}·min ± SD)	ΔP (mm Hg ± SD)	P	Stimulus	Anesthetic
5/10	99 ± 5	391 ± 126	19 ± 7	0.28	AN	Morphine–urethane
5/10	98 ± 9	243 ± 92	16 ± 4		AN + CSN	(3 mg/kg–750 mg/kg)
3/7	105 ± 4	451 ± 218	63 ± 6		5 μg of norepinephrine	
8/15	102 ± 6	562 ± 130	22 ± 3	0.001	AN	Urethane–chloralose
8/15	104 ± 9	257 ± 63	73 ± 15		AN + CSN	(500 mg/kg–50 mg/kg)
7/8	103 ± 6	517 ± 171	73 ± 19		5 μg of norepinephrine	
10/13	99 ± 6	706 ± 299	25 ± 6	0.001	AN	Morphine–chloralose
10/13	99 ± 10	330 ± 135	80 ± 14		AN + CSN	(3 mg/kg–100 mg/kg)
4/4	101 ± 3	698 ± 292	49 ± 2		5 μg of norepinephrine	
5/13	99 ± 7	636 ± 216	20 ± 6	0.001	AN	Sodium–pentobarbital
5/13	95 ± 4	305 ± 51	63 ± 17		AN + CSN	(30 mg/kg)

[a] Number of animals/number of trials.

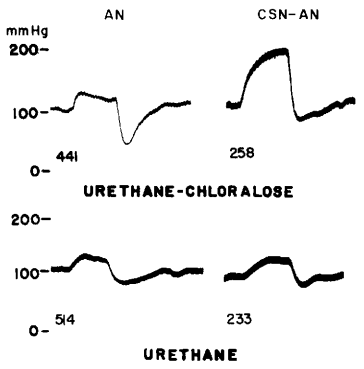

FIG. 1. AN pressor responses in the perfused limbs of dogs anesthetized with urethane–chloralose (top) and morphine–urethane (bottom) before and during CSN stimulation. Initial values of LVR (mm Hg·liter^{-1}·min) are indicated below each response.

smaller in the animals anesthetized with urethane than those seen in the other groups. For this reason, in some cases, it was necessary to either increase the strength or frequency of AN stimulation to obtain comparable control responses. We have considered several possible explanations for the smaller pressor response in these animals. First, the initial value of LVR was lower ($P < 0.05$) in the animals anesthetized with urethane than in the other groups. This fact alone would not be expected to reduce the response to vasoconstrictor influences unless the sensitivity of vascular smooth muscle was also decreased. The pressor responses to intra-arterial injections of norepinephrine into the limbs suggest that the sensitivity to adrenergic stimuli is not less in these animals than in the others (see Table I). We also observed in two animals anesthetized with morphine–chloralose that decreasing LVR to levels similar to those seen during CSN stimulation by infusing nitroglycerine into the limbs did not significantly change the characteristics or the size of the AN pressor response. This suggests that the higher initial level of LVR in the dogs anesthetized with chloralose is not responsible for the larger control response to AN stimulation in these animals. These results further suggest that the reduction in LVR by CSN stimulation is not the sole factor responsible for the augmentation of the AN pressor response.

Cavelo *et al.* (4) and Heistad *et al.* (3) reported that aortic or carotid chemoreceptor stimulation caused vasodilation in cutaneous vessels and vasoconstriction in skeletal muscle. These investigators used a combination of urethane (500 mg/kg) and chloralose (50 mg/kg) as an anesthetic. Although it seemed unlikely, we considered the possibility that urethane anesthesia caused a distribution of blood flow toward the skin and thus reduced the pressure response in the intact limb to AN stimulation. This idea was tested in one animal in which the pressure responses in the perfused skinned and unskinned hind limbs were compared. The responses obtained in the skinned limb to AN stimulation were identical in magnitude and pattern to those observed in the intact limb. Similarly, CSN stimulation caused equivalent depressor responses in both limbs and caused little or no enhancement of the AN pressor response in either limb. From the results above we conclude that the changes in peripheral vascular behavior are probably not responsible for the differences observed in the animals anesthetized with urethane.

Table II summarizes the effect of increasing the level of CSN stimulation on limb perfusion pressure before and during simultaneous stimulation of the AN. In all cases limb perfusion pressure was initially adjusted to about 180 mm Hg before beginning the CSN stimulation series. Stimulation of the AN at this pressure level caused a pressor response of about the same size as that seen at the pressure level of 100 mm Hg. However, after reducing flow to offset the AN pressor response the LVR was not different ($P > 0.05$) from control in any group. Thus, the initial conditions of pressure and LVR at the start of the CSN stimulation series was about the same before and during AN stimulation.

The change in limb perfusion pressure in response to stimulation of the CSN alone was similar ($P > 0.05$) at all levels regardless of the anesthetic used. During stimulation of the AN, CSN stimulation caused less ($P < 0.05$) of a fall in perfusion pres-

sure than that seen during stimulation of the CSN alone in those animals anesthetized with morphine–chloralose, urethane–chloralose, and pentobarbital. The difference between the response curves before and during AN stimulation is maximal at a CSN stimulation level of 4 stimuli/train. This coincides with the level of CSN stimulation at which maximum AN pressor responses were previously observed (1). In the animals anesthetized with morphine–urethane the depressor response curves to CSN stimulation were not changed ($P > 0.05$) by simultaneous stimulation of the AN.

Discussion. In dogs anesthetized with morphine–chloralose, urethane–chloralose, or sodium–pentobarbital stimulation of the CSN depressor reflexes augmented the pressor responses to AN stimulation in the perfused hind limbs. The depressor response curves to graded stimulation of the CSN were also displaced upward by AN stimulation in these dogs. This displacement was greater than that caused by stimulation of the AN alone and therefore cannot be explained on the basis of algebraic summation between these antagonistic reflexes and appears to involve a complex central interaction. We have previously presented arguments, primarily based on the observation that stimulation of the CSN does not augment the pressor response to stimulation of the contralateral AN, that the interaction between these reflexes occurs centrally (1).

The interaction between the AN pressor and the CSN depressor response was not obvious in animals anesthetized with morphine–urethane. The AN pressor response was not augmented by CSN stimulation and the CSN depressor response curve was not shifted upward by AN stimulation. Any reflex interaction that may have occurred appeared to be due to simple summation in these animals.

The animals anesthetized with morphine–urethane had a lower initial level of LVR and responded less to AN stimulation than the other animals. Longnecker and Harris (8) and Altura *et al.* (9) have compared the effects of several anesthetic agents on the microvasculature. The direct vasodepressant effects of the anesthetics used in our study do not appear to differ in a

TABLE II. DEPRESSOR RESPONSES TO CSN STIMULATION BEFORE AND DURING AN STIMULATION

Anesthetic group	*N*	Initial limb pressure (mm Hg ± SD)	Change in limb perfusion pressure (mm Hg ± SD)						Initial LVR (mm Hg·liter^{-1}·min ± SD)	LVD during maximum stimulation of CSN (mm Hg·liter^{-1}·min ± SD)
			CSN stimulation (stimuli/train)							
			2	4	6	8	10	20		
Morphine-chloralose	10									
CSN		183 ± 5	43 ± 23	63 ± 25	73 ± 19	73 ± 18	74 ± 21	78 ± 21	737 ± 110	423 ± 138
CSN + AN		182 ± 4	15 ± 19	22 ± 18	30 ± 16	35 ± 16	37 ± 16	43 ± 14	867 ± 178	662 ± 130
P		0.26	0.01	0.001	0.001	0.001	0.001	0.001	0.06	0.001
Sodium–pentobarbital	5									
CSN		185 ± 2	51 ± 6	80 ± 11	81 ± 11	85 ± 5	86 ± 3	92 ± 8	940 ± 198	478 ± 127
CSN + AN		184 ± 4	20 ± 7	30 ± 11	39 ± 8	44 ± 8	47 ± 8	48 ± 9	956 ± 276	696 ± 178
P		0.74	0.01	0.01	0.01	0.01	0.01	0.01	0.96	0.03
Morphine–urethane	8									
CSN		183 ± 5	35 ± 13	61 ± 17	72 ± 14	77 ± 16	80 ± 16	82 ± 17	497 ± 126	272 ± 67
CSN + AN		179 ± 6	33 ± 13	49 ± 18	64 ± 17	75 ± 18	76 ± 18	78 ± 17	556 ± 147	306 ± 52
P		0.26	0.78	0.22	0.39	0.88	0.64	0.66	0.42	0.30
Urethane–chloralose	8									
CSN		187 ± 6	49 ± 23	65 ± 22	77 ± 22	86 ± 22	88 ± 21	86 ± 21	723 ± 178	388 ± 114
CSN + AN		183 ± 7	12 ± 10	18 ± 14	25 ± 18	34 ± 19	40 ± 24	43 ± 25	770 ± 247	598 ± 191
P		0.60	0.01	0.001	0.001	0.001	0.001	0.01	0.68	0.04

manner sufficient to explain the lower LVR in the urethane-anesthetized dogs. Similarly, the pressor effects of norepinephrine (see Table I) do not suggest that the sensitivity of the microvasculature to adrenergic stimuli is less in these animals than in the other groups.

Reducing initial LVR by infusing nitroglycerine, down to levels comparable to that seen with maximum stimulation of the CSN, did not change the pressor response in two animals anesthetized with chloralose. This observation suggests that the increased AN pressor response during CSN stimulation is not the direct result of the reduction in LVR. This also suggests that the lower initial LVR in the urethane-anesthetized animals is not the sole factor responsible for the smaller AN pressor response in this group. The addition of an anesthetic dose of urethane to an animal anesthetized with morphine–chloralose had little effect on initial LVR or the responses to AN and CSN stimulation. Collectively these observations suggest that the differences observed in the urethane-anesthetized dogs are not the result of a unique, selective interference with some reflex component by urethane. It seems more likely that chloralose and pentobarbital act in some manner that enhances the pressor reflexes.

Early work by Douglas *et al.* (2) and by Neil *et al.* (10) suggested that chloralose and pentobarbital modified the pressor–depressor relationship to stimulation of the CSN in cats. In these studies it was shown that overdosage of chloralose or pentobarbital could reverse the depressor response to strong stimulation of the CSN into a pressor response. The suggested explanation of these results was that these anesthetic agents suppressed the depressor reflexes and allowed a predominance of the pressor reflexes. Overdosage with urethane did not have this effect. Lalley (11) recently reported that the depressor response to stimulation of the CSN, AN, and central vagus nerve is converted to a pressor response in decerebrate cats by the addition of chloralose or pentobarbital but not by urethane. He also suggests that this reversal results from a suppression of the depressor reflexes.

We have previously shown (6) that weak stimulation (0.01 msec, 1–3 V) of the AN at high frequency (100 Hz) caused large depressor responses in dogs anesthetized with morphine–chloralose. Low-frequency (2-Hz) stimulation of these depressor afferents caused minimal depressor responses. We observed similar results in the present experiments in three dogs anesthetized with morphine–chloralose and four dogs anesthetized with urethane. With either anesthetic, minimal depressor responses were seen in response to weak stimulation of the AN at 2 Hz. Thus, it seems unlikely that the small AN pressor responses or the lack of a reflex interaction in the dogs anesthetized by urethane can be explained on the basis that the AN depressor reflexes were greater in these dogs. Similarly, we found no evidence that the CSN depressor reflexes were suppressed more by chloralose or pentobarbital than by urethane.

Collectively our evidence suggests that chloralose or pentobarbital may cause an augmentation of the pressor reflexes rather than suppression of the depressor reflexes. Mancia (12) proposed that the carotid chemoreceptor reflexes may exert part of their vasoconstrictor effects by causing presynaptic inhibition of the carotid sinus baroreceptor depressor reflexes. The neurotransmitter involved in presynaptic inhibition in the spinal cord is generally recognized as γ-aminobutyric acid (GABA). Recent studies have suggested that GABA is also involved as a neurotransmitter in cardiovascular reflexes (13–15). There is growing evidence that pentobarbital and chloralose enhance the action of GABA in the central nervous system (11, 16). Urethane does not appear to have this effect (11). The mechanism responsible for the reflex interaction we have observed may involve synapses which release GABA. This could explain the difference in the results obtained with urethane and chloralose anesthesia. Until more direct evidence is presented that GABA is involved in these reflexes these conclusions must remain highly speculative.

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