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Selective Actions of Intravenous Anesthetics on Nicotinic- and Muscarinic-receptor-mediated Responses of the Dog Adrenal Medulla

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The selective actions of intravenous anesthetics on the cholinergic nicotinic and muscarinic responses of adrenal medullary cells were studied using isolated dog adrenals perfused with modified Locke's solution. Log-probit dose–response curves of the inhibitory effects of the anesthetics on the catecholamine releases induced by acetylcholine, nicotine, and muscarine were determined. Percentage inhibition by the anesthetics at clinically relevant concentrations were 98% of nicotine- and 31% of muscarine-induced releases by alphaxalone 2.6 μM, 76% of nicotine and 13% of muscarine by thiopental 23.9 μM, 86% of nicotine and no inhibition of muscarine by ketamine 17.0 μM, and no inhibition of either response by diazepam 5.0 μM. The ratio of IC_{50} (concentration for 50% inhibition), which was calculated by dividing IC_{50} for muscarine by IC_{50} for nicotine, showed a variety of values ranging from 3.9 for diazepam to 38.0 for ketamine. The results suggest that each anesthetic has characteristic selective inhibitory effects on nicotinic and muscarinic cholinergic responses. The differing effects on the muscarinic responses might be one of the factors contributing to the characteristic properties of each anesthetic, whereas the inhibition of nicotinic responses might reflect a common property for many anesthetics. (Key words: Anesthetics, intravenous: alphaxalone; diazepam; ketamine; thiopental. Receptors: acetylcholine; nicotinic; muscarinic. Sympathetic nervous system: adrenal medulla, catecholamines.)

IT HAS BEEN DOCUMENTED that different anesthetics can produce different spectra of activity in the central nervous system.[1] The multiplicity of effects is possibly due to varying degrees of susceptibility of central synapses to anesthetics. It has been demonstrated in the peripheral cholinergic synapses that halothane and other anesthetics depress the postsynaptic responses to the nicotinic actions of acetylcholine, whereas muscarinic actions are not affected.[2–6]

The present experiments were carried out to examine the effects of intravenous anesthetics on the cholinergic synapse of the adrenal medulla and to determine whether, like volatile anesthetics, at clinical concentrations they have selective actions on nicotinic- and muscarinic-receptor-mediated responses of the dog adrenal medulla.

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Materials and Methods

The details of the procedure have been described previously.[6] In brief, mongrel dogs of either sex weighing 9–13 kg were anesthetized with sodium pentobarbital (30 mg/kg iv or ip). Both adrenals were exposed through a midline abdominal incision and isolated outside the body together with the adrenolumbar vein. The adrenolumber vein was cannulated, and the glands were perfused retrogradely at a pressure ranging from 45 to 80 cmH_2O with a warmed (37°C) modified Locke's solution aerated with 95% O_2 and 5% CO_2. The solution was composed as follows (in mM): NaCl 154, KCl 5.6, $CaCl_2$ 2.2, glucose 10, and tris-HCl buffer 40; *p*H 7.4. Perfusion was carried out at a constant rate in each experiment, ranging from 0.8 to 1.3 ml/min. About 45 min were allowed to elapse before any treatment in order to achieve equilibrium. The adrenals were stimulated three times with one of the agonists, *i.e.*, acetylcholine, 20 μM, nicotine, 5 μM, and muscarine, 20 μM. The stimulation period was 1 min, followed by 20-min recovery intervals. The agonists and test anesthetic drugs were dissolved in Locke's solution and administered by continuous infusion by switching a valve on the tubing leading to the glands.

The effluent from the adrenals was collected into glass tubes kept on ice at 1-min intervals starting 1 min prior to the stimulation and lasting for 4 min, and catecholamine content was measured by the trihydroxyindole method[7] without further purification on alumina. In most instances, a 0.2-ml aliquot with 1.0 ml of 1 M acetate buffer (final *p*H 6.3) was used for the assay. This assay method has a limit of sensitivity of 2 ng of each catecholamine. The interassay and intraassay variations were less than 5%. In all cases, it was ascertained that the drugs used did not interfere with the assay. Stimulant-induced catecholamine release was calculated as the difference between spontaneous catecholamine release and release during stimulation.

In controls, the adrenals were stimulated in the absence of anesthetics. In the experiments performed to examine the effects of intravenous anesthetics, the adrenals were perfused with a solution containing the anesthetics during a period starting 10 min prior to the second stimulation and lasting until 3 min after the second stimulation. The percentage of inhibition of catecholamine release by the

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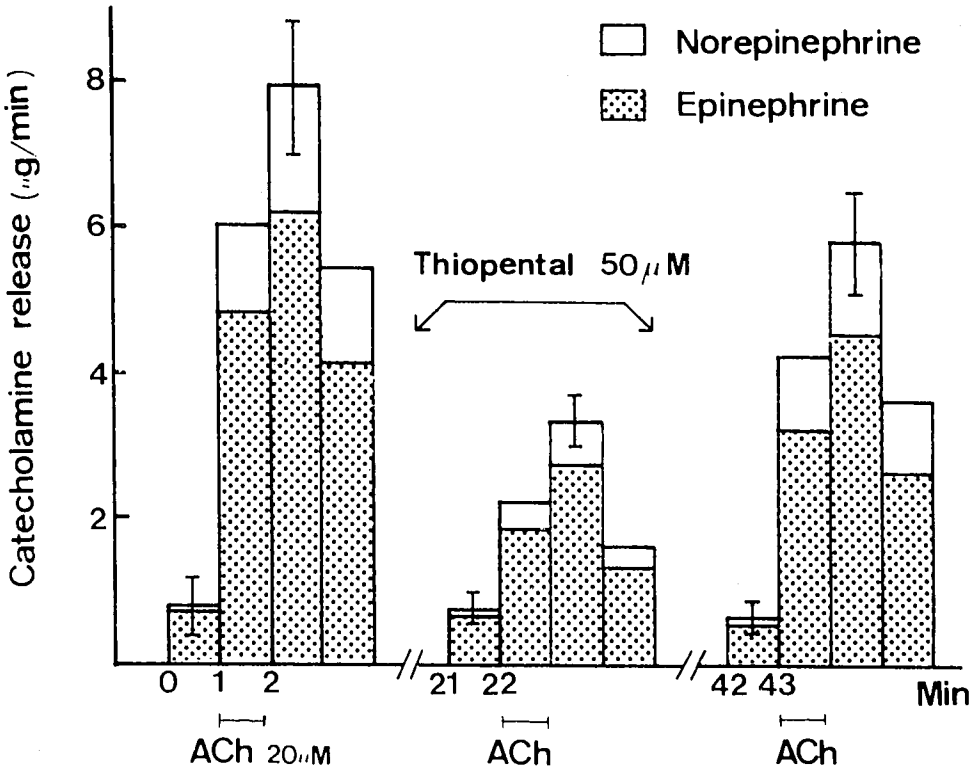

FIG. 1. Time course of the experiments to examine the effect of anesthetics on the agonist-induced catecholamine release from the adrenal medulla. The ordinate represents the rate of catecholamine release during a 1-min period (n = 4, mean ± SE for total catecholamines). Isolated dog adrenals were perfused retrogradely with a modified Locke's solution at 37°C and stimulated three times with acetylcholine. The stimulation period was 1 min, followed by 20-min recovery intervals. Thiopental was added to perfusate during the period from 10 min prior to the second stimulation and lasting until 3 min after the second stimulation.

anesthetics was calculated by the decrease in the ratio of the second response to that of the first in comparison with the control condition.[6] The anesthetics used were alphaxalone (0.05–20 μM), diazepam (10–500 μM), ketamine (1–200 μM) and thiopental (2–200 μM). The dose range of each anesthetic was that determined by preliminary study to reduce the catecholamine release induced by the agonists to between 10 and 90% of control; at least three experiments were carried out for each dose.

The probit method[8] of statistical analysis was used for calculation of dose–response curves. Per cent inhibitions were converted into probit values and plotted against a log of the doses. The IC_{50} (concentration of inhibitor required to depress the response by 50%) and the per cent inhibition by a given clinical concentration of the anesthetic were calculated by the probit analysis.[8]

Results

Spontaneous release of catecholamines during the 1-min period prior to stimulation amounted to 0.7 ± 0.3 μg/min (mean ± SE, n = 6). In controls, acetylcholine-induced catecholamine release during the 3-min collection period on the first stimulation was 17.9 ± 2.1 μg (n = 6); the amounts released on the second and third stimulations were 83.4% ± 3.1% and 73.1 ± 2.7%, respectively, of that released during the initial stimulation. Similarly, initial releases by nicotine and muscarine were 19.4 ± 3.1 μg (n = 5) and 17.4 ± 2.2 μg (n = 5), respectively, and the amounts released on the second stimulation were 84.5 ± 3.3% and 82.1 ± 2.3%, respectively. Figure 1 shows the time course of the experiment and the effect of 50 μM thiopental on acetylcholine-induced catecholamine release. Acetylcholine-induced catecholamine release was inhibited by thiopental reversibly, because the release on the third stimulation without thiopental was restored to the control level (approximately 75% of the initial stimulation).

Figure 2 shows the dose–response curves for the inhibitory effects of the anesthetics on catecholamine release induced by acetylcholine, nicotine, and muscarine. Each anesthetic inhibited the adrenal response to these agonists, but the ability of the anesthetic to inhibit catecholamine release induced by muscarine was less than its activity in depressing the release induced by nicotine. The ability to inhibit the response to acetylcholine was between those to inhibit the responses to nicotine and muscarine. The inhibitions of catecholamine release by these anesthetics were reversible, since the amount of catecholamines released on the third stimulation without anesthetics were restored to the control level (data not shown).

Table 1 shows the effects of the anesthetics at clinical concentrations on nicotine-, acetylcholine-, and muscarine-induced catecholamine release. The clinical concentrations were determined on the basis of the literature.[9–13] All of the anesthetics except for diazepam ex-

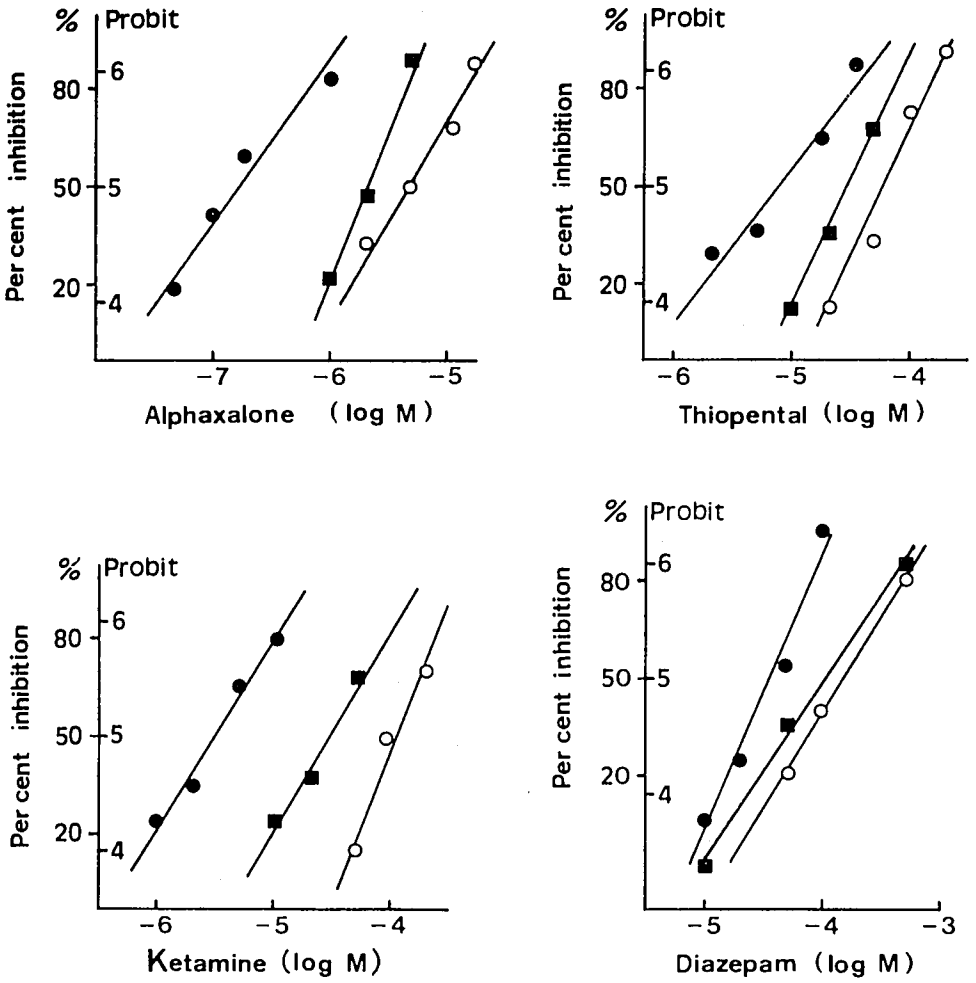

FIG. 2. The dose–response curves on a log-probit plot with respect to the inhibitory effects of intravenous anesthetics on the agonist-induced catecholamine release from the adrenal medulla. The adrenals were stimulated three times with one of the agonists, *i.e.*, nicotine 5 μM (●), acetylcholine 20 μM (■), and muscarine 20 μM (○). The second stimulations were performed in the presence or absence of anesthetics, and per cent inhibitions were calculated by the decrease in the ratio of the second response to the first response in comparison with the control condition. Per cent inhibitions were converted into probit values and plotted against the log of the concentrations. The linear regression lines were obtained according to the method of Finney.[8] Each point represents the value determined by at least three experiments.

erted marked inhibitory effects on the nicotinic response, but the degrees of inhibition of muscarinic responses were markedly different among the anesthetics tested. Diazepam at a clinical concentration (5 μM) had no effect on any of the responses to nicotine, acetylcholine, and muscarine.

TABLE 1. Effects of Intravenous Anesthetics at Clinical Concentrations* on Nicotine-, Acetylcholine-, and Muscarine-induced Catecholamine Release

Anesthetics		Inhibition of Catecholamine Release (%)†		
		Nicotine	Acetylcholine	Muscarine
Alphaxalone	2.6 μM	98	58	31
Thiopental	23.9 μM	74	45	13
Ketamine	17.0 μM	86	34	0.1
	3.7 μM	56	0.6	0
Diazepam	5.0 μM	0.2	0	0

* The clinical concentrations were determined on the basis of the literature.

† Per cent inhibition was calculated by probit analysis.

Table 2 shows the concentrations needed for 50% inhibition (IC_{50}) of the responses to nicotine and muscarine. The ratio of IC_{50} showed a variety of values ranging from 3.9 for diazepam to 38.0 for ketamine.

Discussion

Anesthetics at concentrations that exceed clinical applicability are very unselective in their actions and can affect a variety of cellular functions. Therefore, in any discussion of the mode of action of anesthetics, it is important to relate the effects produced by a given concentration of an anesthetic to that required to maintain anesthesia. Whereas the potency of inhalation anesthetics is commonly determined by measuring MAC, there is some difficulty in determining the potency of intravenous anesthetics.[14] However, the same concept as MAC has been applied to the plasma concentrations of various intravenous agents,[15] and the plasma concentrations necessary for clinical anesthesia as well as the fraction bound

to plasma protein have been determined for many of these drugs.

Recently, Becker[11] determined the plasma levels of thiopental necessary in humans for loss of trapezius muscle response. This occurred at a free plasma thiopental concentration of 6.3 μg/ml. This is equivalent to 23.9 μM, and, in the present study, this was referred to as a clinical concentration. Simpson[12] measured the plasma concentrations of alphaxalone in patients administered Althesin®, 60 μl/kg iv. The free fraction associated with anesthesia was estimated as 1.8–2.7 μM. In the present study, 2.6 μM was referred to as a clinical concentration. Cohen *et al.*[9] found that, in rats anesthetized with ketamine, the recovery of the righting reflex occurred at a free plasma fraction of 17.0 μM. Wieber *et al.*[10] reported in humans that the free plasma concentration of ketamine 15 min after intravenous injection of 2.5 mg/kg was about 3.7 μM. In the present study, both 17.0 μM, and 3.7 μM were referred to as clinical concentrations. Dundee[13] found that the anesthetic dose of diazepam varied from 0.2 to 1.8 mg/kg iv and that the plasma diazepam concentration 1 h after intravenous administration of 1 mg/kg was about 1,000–1,200 ng/ml (3.5–4.2 μM). Although the fraction was unknown, in the present study, 5 μM was referred to as a clinical concentration.

Our results show that all of the anesthetics tested except for diazepam exerted marked inhibitory effects on the nicotinic response at clinical concentrations, while muscarinic responses were affected slightly or not at all. A similar selectivity of actions of halothane,[2,4] ketamine[3] and pentobarbital[5] have been demonstrated previously in the sympathetic ganglia, although clinical concentrations always were not used. These findings seem to confirm the susceptibility of nicotinic responses of cholinergic synapses to many volatile and intravenous anesthetics.

Contrary to the nicotinic responses, the degrees of inhibition of muscarinic responses were markedly different among the anesthetics tested. Marked differences among the effects of anesthetics on the muscarinic responses also have been reported in the guinea pig olfactory cortex by Smaje.[16] These findings suggest that the differing effects on the muscarinic responses might be one of the factors involved in the specific properties of each anesthetic, whereas the inhibition of nicotinic responses might contribute to the common properties of anesthetics.

In contrast with the other anesthetics tested, diazepam at anesthetic concentrations had no effect on either the nicotinic or muscarinic responses. The concentrations for 50% inhibition of nicotinic and muscarinic responses deviated from the clinical range. These results suggest that the effect of diazepam on the cholinergic excitatory synapse of the adrenal medulla would be a nonspecific action having no relation to anesthesia. This finding might be in accordance with the fact that specific receptors for benzodiazepines have been identified in brain tissue and the mechanisms of diazepam action are currently attributed to increased inhibitory neurotransmission.[17]

TABLE 2. Selectivity of Inhibitory Effects of Intravenous Anesthetics on Nicotine- and Muscarine-induced Catecholamine Release

	Concentration for 50% Inhibition (μM ± SE)*		Ratio of IC_{50}† (Muscarine/ Nicotine)
	Nicotine	Muscarine	
Alphaxalone	0.17 ± 0.02	5.01 ± 0.32	29.5
Ketamine	2.95 ± 0.14	112.20 ± 5.39	38.0
Thiopental	7.76 ± 0.68	70.79 ± 4.16	9.1
Diazepam	35.50 ± 3.21	138.04 ± 9.55	3.9

* Concentration for 50% inhibition (IC_{50}) was calculated by the probit analysis.

† The ratio of IC_{50} was calculated by dividing IC_{50} for muscarine by IC_{50} for nicotine.

In the adrenal chromaffin cells, fast Na^+ channels do not appear to play an essential role in acetylcholine-induced catecholamine release.[6] In a previous study,[6] we examined the mechanisms involved in the differential effects of halothane on nicotinic and muscarinic responses and advanced the hypothesis that there might be a different susceptibility to halothane between the Ca^{++} channels that were linked to the respective nicotinic and muscarinic receptors. It seems possible that the same mechanism might be involved in the actions of intravenous anesthetics. Furthermore, the present results have shown that each anesthetic has a characteristic selectivity of action on the nicotinic- and muscarinic-receptor-mediated responses. This selectivity possibly suggests that the anesthetic molecule occupies a critical domain in these receptor proteins, each agent having its own characteristic impact upon these proteins, resulting in characteristic perturbation of each receptor-linked Ca^{++} channel.

In conclusion, the nicotinic response of the dog adrenal medulla has marked suspceptibility to many intravenous anesthetics, except for diazepam, at clinical concentrations, whereas the degrees of inhibition of muscarinic responses are markedly different among the anesthetics tested. The findings suggest that each anesthetic has a characteristic selectivity of inhibitory effects on nicotinic and muscarinic cholinergic responses and that the effects on the muscarinic responses might be one of the factors involved in determining the characteristic properties of each anesthetic, whereas the inhibition of nicotinic responses might contribute to the common properties of many anesthetics.

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