Central Stimulatory Activity of Vanillic Diethylamide

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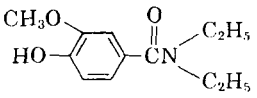
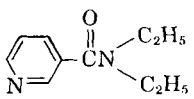
Vanillic diethylamide produced central nervous system effects indicative of medullary stimulation when injected into frogs and rats. Vanillic diethylamide administered intravenously to anesthetized dogs and cats produced a marked increase in both the rate and depth of respiration, which was usually accompanied by the return of the corneal reflex and a mild, transient hypotensive effect. The transient hypotensive effect could be eliminated by administering the compound via a slow infusion at rates ranging between 1 and 8 mg./Kg./min. The compound produced identical responses prior to and after bilateral carotid sinus area denervation, and it appears that the compound acts mainly on the medullary centers and that the carotid sinus-body complex is not essential for the stimulatory activity. A 10 mg./Kg. quantity of vanillic diethylamide administered into the arterial inflow to the recipient's head in 2 dog cross-circulation experiments awakened the recipient animal and produced a transient pressor effect in the recipient.

IT HAS BEEN known for many years that the analeptic activity of the alkylated acid amides was due to the alkylation of the nitrogen of the acid amide group (1). The clinical success with nikethamide, an active member of the alkylated acid amide group, prompted further synthesis of related compounds. Vanillic diethylamide, 3-methoxy-4-oxybenzoic acid-diethylamide (see Fig. 1), was prepared and tested on experimental animals in an attempt to produce a more potent compound (2, 3). The object of the present study was to investigate certain central stimulatory actions of the compound and to study its possible sites of action.



Vanillic Diethylamide



Nikethamide

Fig. 1.—Chemical structure of vanillic diethylamide and nikethamide.

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EXPERIMENTAL PROCEDURES

Preliminary CNS Screening in Frogs.—Doses of vanillic diethylamide, ranging between 10 and 50 mg./Kg., were administered into the ventral lymph sac of 12 frogs. General activity was noted and if hyperreflexia or convulsions occurred, either decerebration or decerebration and demedullation was performed.

Preliminary CNS Screening in Rats.—An aqueous solution of vanillic diethylamide was administered intraperitoneally to 16 rats in doses ranging from 25 to 75 mg./Kg., and the gross behavior of the animals recorded.

Effect on Anesthetized Dogs and Cats.—Mongrel dogs were anesthetized with an intravenous dose of 35 mg./Kg. of sodium pentobarbital. Cats were anesthetized by an intraperitoneal injection of the same dose of sodium pentobarbital. Blood pressure was recorded from a carotid or femoral artery via a Statham transducer, and respiration was recorded from a thermocouple wire in the tracheal cannula. The neural plexi surrounding both carotid sinuses and carotid bodies were stripped in three experiments. The elimination of the pressor response to bilateral carotid occlusion was utilized as one of the criteria for complete denervation of the carotid sinus area. Physiological responses were recorded on a Grass polygraph. Fresh aqueous solutions of vanillic diethylamide were administered intravenously in doses ranging from 2.5 to 25 mg./Kg.

Effect on Dogs and Cats under Severe Barbiturate Depression.—Dogs and cats were anesthetized and prepared for the recording of blood pressure and respiration as previously noted. The animals were further depressed by intravenous doses of sodium pentobarbital. At a point of severe respiratory depression, vanillic diethylamide was administered intravenously in an aqueous solution.

Effect of Vanillic Diethylamide Infusion on Depressed Cats.—Cats were anesthetized by an intraperitoneal injection of 35 mg./Kg. of sodium pentobarbital. Physiological recordings were made as previously noted. Vanillic diethylamide was infused intravenously into the depressed animal utilizing a microdrip apparatus.[1] Bilateral carotid

[1] Venopak microdrip, Abbott Laboratories.

sinus-body denervation was produced in three preparations.

Central Effects of Vanillic Diethylamide.—The central effects of vanillic diethylamide were investigated in two dog cross-circulation preparations. Mongrel dogs were anesthetized by an intravenous injection of pentobarbital sodium, 35 mg./Kg., and the vascularly isolated, neurally intact recipient head preparation was obtained utilizing the procedure described by Buckley, *et al.* (4). Donor and recipient femoral blood pressures were obtained, and electroencephalographic tracings were obtained from frontal electrodes in the recipient. Vanillic diethylamide, 10 mg./Kg., was administered in aqueous solution via the carotid artery to the recipient's head.

RESULTS

Preliminary CNS Screening in Frogs.—Doses of 10 and 25 mg./Kg. of vanillic diethylamide produced hyperreflexia which disappeared upon decerebration. Injection of 50 mg./Kg. produced clonic convulsions which were abolished by demedullation.

Preliminary CNS Screening in Rats.—The effects of vanillic diethylamide, in doses ranging between 25 and 75 mg./Kg., i.p., on the activity of albino Wistar rats are summarized in Table I and indicate that the compound is a potent stimulant of the central nervous system. The type of convulsions produced by the larger doses suggests the medulla as the possible site of stimulation.

TABLE I.—EFFECTS OF VANILLIC DIETHYLAMIDE ON ALBINO RATS

No. of Animals	Dose, i.p., mg./Kg.	Results
3	25	No effect
9	50	2 showed slight stimulation, 4 showed extreme excitement short of convulsions, 3 showed clonic convulsions and death within 5 minutes
4	75	3 showed mixed convulsions and death within 5 minutes, 1 showed slight stimulation

Effect on Anesthetized Dogs and Cats.—The intravenous administration of vanillic diethylamide, in the dose range of 10 to 25 mg./Kg., to dogs and cats produced general signs of central nervous system stimulation (See Table II). The common picture of initial apnea lasting 60 seconds or less followed by an increased rate and/or depth of respiration was observed in most of the experiments. The initial hypotensive effect followed by the return to the predrug level or slightly above that level was also seen in the majority of the experiments. Doses of 20 and 25 mg./Kg., i.v., into cats produced signs of arousal. The intravenous injection of vanillic diethylamide to three dogs having bilateral denervation of the carotid sinus-body complex produced the same physiological responses that were seen in animals having intact sinus areas. The administration of 10 mg./Kg. of vanillic diethylamide to the first dog produced a transient fall in blood pressure from 245/135 mm. Hg to 180/80 mm. Hg with a return to control levels within 2 minutes. The respiration was increased, following a transient apnea, to 17 per minute from an initial rate of 13 per minute. The blood pressure in the second dog showed a transient fall from 250/128 mm. Hg to 150/80 mm. Hg and a recovery to 195/120 mm. Hg within 80 seconds following the intravenous injection of 15 mg./Kg. of vanillic diethylamide. The respiration, following a short period of apnea, increased to 18 per minute from the control value of 4 per minute; the respiratory rate then slowed gradually to 7 per minute. The third dog showed an increase in respiratory rate to 63 per minute from the control level of 47 per minute following 10 mg./Kg. of the drug, i.v. The blood pressure followed the same pattern as was seen in the previous dogs.

Effect on Dogs and Cats under Severe Barbiturate Depression.—Vanillic diethylamide, in doses from 5 to 25 mg./Kg., produced in all experiments but one an increase in the rate of respiration (See Table III). Doses of 25 mg./Kg., i.v., in the cat generally produced signs of arousal from the anesthesia. Many of the cardiovascular responses were that of initial transient hypotension with a recovery to near normal levels of blood pressure within a few minutes.

Effects of Vanillic Diethylamide Infusion on Depressed Cats.—The administration of vanillic

TABLE II.—EFFECT OF VANILLIC DIETHYLAMIDE ON ANESTHETIZED[a] DOGS AND CATS

Animal	Initial Blood Pressure, mm. Hg	Initial Respiration, rate/min.	Dose, i.v., mg./Kg.	Effects	
				Respiration	Blood Pressure,[b] mm. Hg
Dog	190/135	18	2.5	17/min. with increased depth	205/145
Dog	250/140	72	10.0	Rate drop to 66/min., depth increased by 50%	Transient fall, then maintained at 260/150
Dog	130/75	31	10.0	Short period of apnea followed by increased rate and depth	Transient fall and then recovery
Dog	175/115	28	10.0	Initial apnea, then increased to 36/min.	Transient fall, followed by a slight hypertensive effect
Dog	200/120	5	15.0	Apnea for 60 sec., rate increased to 12/min., then back to normal in 4 min.	Fall to 104/42, then rise to 175/100 in 80 sec.
Cat	175/130	24	5.0	No change	No change
Cat	75/55	12	10.0	Transient increase	No change
Cat	193/140	19	20.0	Increased to 40/min.	Increased 25%, animal awoke
Cat	145/100	26	25.0	Increased to 50/min.	No change, motor activity of limbs

[a] Anesthetic—pentobarbital sodium, 35 mg./Kg., i.v., for dogs, i.p., for cats. [b] Motor activity also noted.

TABLE III.—EFFECT OF VANILLIC DIETHYLAMIDE ON DOGS AND CATS UNDER DEEP PENTOBARBITAL DEPRESSION

Animal	Additional Doses of Sodium Pentobarbital,[a] mg./Kg.	Blood Pressure, mm. Hg	Respiration, rate/min.	Dose, i.v., mg./Kg.	Effects	
					Blood Pressure, mm. Hg	Respiration,[b] rate/min.
Dog	22.5	155/105	2	10.0	Transient fall then rise to 175/120	Increased to 33
Cat	15.0	...	2–3	5.0	Transient fall then increased to 180/125	Increased to 11 with increase in depth
Cat	7.5	100/75	Stopped	5.0	Increased to 175/125	Increased to 10
Cat	11.0	128/80	20	6.0	Fall to 100/68	Transient apnea, then 15
Cat	9.0	147/108	15	10.0	Transient fall to 115/53 then returned to 151/113 in 3 min.	Increased to 21 for 1 min., then returned to 15
Cat	6.5	...	20	25.0	.....	Extremely rapid with convulsions
Cat	15.0	75/60	Stopped	25.0	160/100	Increased to 15 for 5 min., then stabilized at 8, then corneal reflex positive
Cat	18.0	...	2–3	25.0	Increased	Increased to 15

[a] Dogs anesthetized with pentobarbital sodium, 35 mg./Kg., i.v.; cats anesthetized with pentobarbital sodium, 35 mg./Kg. i.p. [b] Motor activity also noted.

TABLE IV.—EFFECT OF THE INTRAVENOUS INFUSION OF VANILLIC DIETHYLAMIDE ON ANESTHETIZED CATS[a]

Animal	Infusion Rate, mg./Kg./min.	Total Dose, mg./Kg.	Time, min.	Blood Pressure, mm. Hg	Respiration, rate/min.	Motor Activity
A	1.0	89.0	0	200/135	8	.....
			43[b]	240/165	12	.....
			60	...	..	Head movements
			89[c]	225/150	15	Corneal reflex
			99	...	14	.....
			109	...	..	Corneal reflex negative
			129	190/130	13	.....
B	7.8	80.8	0	138/100	17	.....
			6	Irregular	20	Ear twitch
			8.6	Irregular	7.8	Wink reflex
			10.2[c]	Irregular	..	Corneal reflex positive
			12.0	200/120	..	Convulsion
			17.0	...	..	Corneal reflex negative
			30.0	175/125	9	.....
C	3.5	36.2	0	104/68	8	.....
			5[b]			
			10.3[c]	140/83	Slowed and irregular	Ear twitch
			17	175/100	10	.....
D[d]	1.8	56.9	0	172/125	15	.....
			10[b]	194/125	17	Ear twitch
			25	...	12	Corneal reflex positive
			31.6[c]	160/195	9	.....
			35	...	..	Corneal reflex negative
			50	184/121	15	.....
E[d]	1.8	61.7	0	130/90	Slowed and irregular	Wink reflex positive, corneal reflex negative
			8	140/95	8	Corneal reflex positive
			14[b]	160/100	..	Ear twitch
			34.3[c]	123/75	25 shallow	.....
			42	160/92	Slowed with greater depth	.....
F[d]	1.0	30.0	0[b]	205/145	7	.....
			7	Falling	8	Wink reflex positive
			16	Falling	9	Ear twitch
			18	Falling	9	Corneal reflex positive
			30[c]	175/125	7–8	.....
			35	175/125	15	Ear twitch stopped

[a] Anesthetic, pentobarbital sodium, 35 mg./Kg., i.p. [b] Peak hypertensive effect. [c] Cessation of infusion. [d] Bilateral carotid sinus-body denervation.

diethylamide by slow infusion produced pronounced CNS stimulatory effects in anesthetized cats, even when the carotid sinus-body complexes were denervated (See Table IV). In most cases, the blood pressure was elevated during initial stages of the infusion and then gradually fell after a period of time. When the infusion was stopped, arterial blood pressure usually increased or remained stable. The

peak hypertensive response usually occurred very close to the onset of visible motor movements. Since the arterial pressure began to drop shortly after motor activity was observed, it is entirely possible that pain stimuli might be responsible for this secondary depressor response. The respiratory rate generally increased during the initial phase of the infusion and then gradually decreased. The rate of respiration immediately increased when the infusion was stopped. The infusion of vanillic diethylamide into denervated cats produced the same effects as those seen during the infusion of the drug into cats having intact sinus areas (See Table IV).

Central Effects of Vanillic Diethylamide.—The administration of 10 mg./Kg. of vanillic diethylamide into the arterial inflow to the recipient's head in two cross-circulation experiments produced nearly complete arousal of the animals. The recipient animals blinked their eyes, wagged their tails, and attempted to move their heads. An initial apnea occurred in the recipient dogs which was followed by a resumption of respiration that was thoracic in nature as compared to the abdominal breathing observed prior to drug administration. The compound produced an initial hypertensive effect in the recipient dogs. There was little change in the blood pressure and respiration of the donor dogs although there was a slight transient hypotensive response in one dog. An electroencephalogram, obtained from frontal electrodes in the recipient dog, illustrated an increased activity immediately after the administration of vanillic diethylamide. Major analeptic effects were not seen in the donor dog.

DISCUSSION

The parenteral administration of vanillic diethylamide into frogs, rats, cats, and dogs produced definite signs of central nervous system stimulation. Injection of 50 mg./Kg. of the drug into the ventral lymph sac of frogs produced clonic convulsions which were abolished by demedullation, while 50 and 75 mg./Kg.-doses injected in rats produced tonic-clonic convulsions. Single doses of 5 to 25 mg./Kg. administered intravenously into anesthetized and deeply anesthetized dogs and cats generally produced a decrease in the depth of anesthesia. Following the injection of the drug, an initial phase of apnea and hypotension usually occurred; the respiration then increased in rate and/or depth while the blood pressure returned to normal or slightly hypertensive levels. Nikethamide is believed to stimulate respiration through stimulation of the carotid body (5). It has also been reported that vanillic diethylamide acts through stimulation of this receptor area (6); however, single and infused doses of vanillic diethylamide following bilateral denervation of the carotid body-sinus area produced similar effects to those seen prior to denervation. It would appear that the carotid body has little, if any, part in the mechanism of action of the compound.

The infusion of vanillic diethylamide into depressed cats decreased the depth of anesthesia in accordance with the rate of infusion. It would appear that this method of administration might lend itself well to clinical control of overdepressed patients, especially since vanillic diethylamide appears to be quickly metabolized.

The hypotensive effect of the compound produced by single intravenous injections into depressed animals was not observed following intra-arterial injection into the recipient animal in cross-circulation experiments. A hypertensive effect rather than the expected hypotensive response occurred in these animals. This might indicate that the hypotensive response was due to peripheral mechanisms rather than effects mediated through the central nervous system.

It appears that vanillic diethylamide is a potent central nervous system stimulant having primary effects on the medullary neural receptors with little or no effect taking place through the carotid body chemoreceptors or the carotid sinus receptors. It should also be noted that the cat appears to be extremely sensitive to the drug.

SUMMARY

1. The central stimulatory effects of vanillic diethylamide were studied in frogs, rats, dogs, and cats.

2. Vanillic diethylamide produced central stimulatory effects in all species tested.

3. The stimulatory action of vanillic diethylamide appears to be mainly due to direct stimulation of medullary receptor sites and not to stimulation of the carotid body-sinus complex.

4. Large doses of the compound produced transient hypotensive effects which could be eliminated by administering vanillic diethylamide by slow intravenous infusion.

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