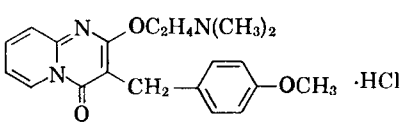
2-(2-Dimethylaminoethoxy)-3-(4-methoxybenzyl)-4H-pyrido[1,2-*a*]pyrimidin-4-one HCl (CL-1182C), A Potent Histamine Releaser

By THAD P. PRUSS and JOHN HIDALGO

CL-1182C, a pyridopyrimidine, exhibited a nonspecific spasmolytic activity on the isolated ileum. On oral and intravenous administration to nonanesthetized dogs, the compound caused salivation, vomiting, violent propulsive defecation, and seizures. An intravenous dose of 1 mg./Kg. in anesthetized dogs produced a pronounced fall in arterial pressure and an increase in respiratory rate. Measurement of femoral blood flow and peripheral pressures indicated that the hypotension was associated with a fall in peripheral resistance. A fall in systolic pressure also was noted when 100 mg./Kg. of CL-1182C was given intragastrically to nonanesthetized dogs. The histamine release activity of CL-1182C was confirmed by cross tachyphylaxis of the vasodepressor responses to 48/80 and by increased plasma histamine levels.

FOR MANY YEARS, histamine release was thought to be associated primarily with anaphylaxis (1). Then, in 1951, Paton (2) discovered that compound 48/80, a condensation product of *p*-methoxyphenylethylmethylamine and formaldehyde, was a potent histamine releaser in rats. In 1955, polymyxin-B also was shown to be a potent histamine releaser (3). Both 48/80 and polymyxin-B are relatively large molecules, the former being a mixture of polymers of undetermined molecular weight and the latter a polypeptide. Histamine release has also been described for smaller molecules, such as morphine and octylamine, although they have a lower order of potency (4, 5). This paper describes the experiments leading to the discovery of another potent histamine releaser, CL-1182C.[1]



EXPERIMENTAL

Spasmolytic Activity.—The *in vitro* spasmolytic activity of CL-1182C was studied by the method of Burn (6). The isolated rabbit ileum was used for determining antagonism to acetylcholine and barium chloride and the guinea pig ileum for antiserotonin and antihistamine activity.

Cardiovascular Activity.—Dogs were anesthetized with sodium thiopental 20 mg./Kg. intravenously, and maintained with chloralose, 60 mg./Kg. intravenously. One femoral vein was catheterized for intraveneous injection. A femoral artery was catheterized and arterial pressure measured by a Statham pressure transducer recording on a Gilson polygraph. Respiration was recorded by means of a thermistor probe.

Received July 13, 1964, from the Research Division, Cutter Laboratories, Inc., Berkeley, Calif.
Accepted for publication March 3, 1965.
Presented to the Western Pharmacology meetings, 1964.
[1] Synthesized by Dr. R. E. Allen, Organic Chemistry Department, Cutter Laboratories, Inc.

Femoral blood flow was determined by shunting the blood from a femoral artery through a Shipley-Wilson blood flow meter, then returning the blood to the femoral artery. Injections of the test compound were made intra-arterially into the tubing distal to the rotameter. The volume of solution was kept at 0.25 ml.

Changes in peripheral resistance were measured by shunting the blood from both femoral arteries through a T6-S Sigmamotor pump back into the femoral arteries. A pressure transducer was inserted into the system between the pump and the return to the femoral arteries. Blood was returned at a constant flow and at a pressure approximating systemic arterial pressure. A fall in pressure in the system indicates a decrease in peripheral resistance.

Histamine Determination.—Plasma histamine was determined by the method of Shore *et al.* (7). A catheter was inserted retrograde into one femoral artery of an anesthetized dog and its tip located approximately in the abdominal aorta. Five blood samples of 10 ml. each were withdrawn and served as a control. The blood was replaced by 50 ml. of 5% glucose–0.9% saline. After injection of the test compounds, 10-ml. blood samples were withdrawn at 1, 10, and 20 min. postinjection and analyzed for histamine. This experiment was conducted in four dogs.

Drug Administration.—When the test compound was administered intragastrically by stomach tube, a 10% drug solution was used, followed by 10 ml. of water.

The compound was administered intravenously as a 1% solution.

RESULTS

CL-1182C inhibited contractions of the isolated ileum induced by acetylcholine, histamine, serotonin, and barium chloride, indicating that the compound has a papaverine-like nonspecific spasmolytic activity. Paton describes this same type of nonspecific spasmolytic activity for 48/80 (4).

A list of the effects noted when CL-1182C was administered to nonanesthetized dogs is summarized in Table I. Many of these same signs were reported by Paton (4) following the administration of 48/80 to cats and dogs. Since CL-1182C produces convulsions, this suggests that the compound enters the central nervous system.

TABLE I.—EFFECTS BY CL-1182C IN NONANESTHETIZED DOGS

Dose, mg./Kg.	Administration Route
	Intragastric
100	Vomiting, retching, ataxia, jerky movements, salivation
200	Vomiting, violent propulsive defecation
	Intravenous
2.5	Vomiting, defecation, transitory collapse
5.0	Seizure, defecation, collapse, violent retching

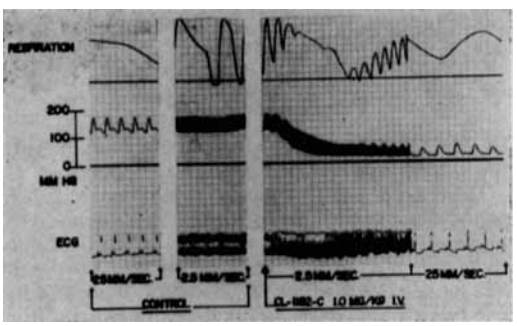


Fig. 1.—Effect of CL-1182C on arterial pressure, respiration, and electrocardiogram of an anesthetized dog.

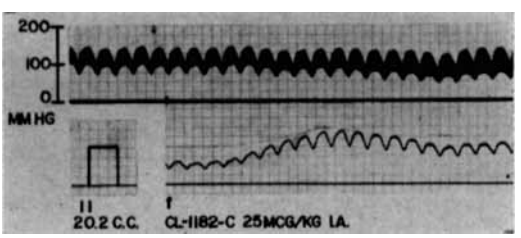


Fig. 2.—Effect of CL-1182C on femoral blood flow. The upper tracing is systemic arterial pressure; the lower tracing is femoral blood flow. A calibration response is shown at the lower left.

CL-1182C, injected intravenously into anesthetized dogs, produced a pronounced fall in arterial pressure, increased respiratory rate, and depressed the S-T interval of the ECG (Fig. 1). This ECG change may have been the result of the hypotension.

In an attempt to elucidate the mechanism for the vasodepressor response, CL-1182C was studied for its effect on femoral blood flow in five dogs. (See under *Experimental.*) The results of a typical experiment are presented in Fig. 2. CL-1182C, after intra-arterial administration, caused a significant increase in femoral blood flow. This increase in flow was due to vasodilation in the femoral area.

To determine whether this vasodilation would occur after intragastric administration of CL-1182C, hind limb flow and pressure were kept constant in anesthetized dogs by a Sigmamotor pump. The data in Table II indicate that doses of 100 mg./Kg. of CL-1182C caused a fall in the peripheral pressure and systemic arterial pressure in two dogs; a dose of 50 mg./Kg. produced a proportional fall in both pressures in two out of three dogs. In all the experiments, there was a fairly rapid onset of effect. The data suggest that the vasodepressor response of the compound was associated with a decrease in peripheral resistance.

The hypotensive response to intragastric CL-1182C also manifests itself in nonanesthetized dogs. The results are summarized in Table III. The pressures listed were taken at the peak of the response by a Decker caudal plethysmograph. In all four dogs, CL-1182C, given intragastrically in a dose of 100 mg./Kg., caused a significant fall in systolic pressure. As a control, water in volumes comparable to those employed for drug administration produced no significant effect.

The previous experiments suggested that histamine was being released. The following experiments verified that histamine was responsible for the effects noted.

The vasodepression produced by CL-1182C becomes tachyphylactic when injected at hourly intervals. The compound also produces cross tachyphylaxis with 48/80; Fig. 3 shows results of typical experiments. This experiment was conducted in three dogs. Neither 48/80 nor CL-1182C produced any significant effect after tachyphylaxis to the other. The results indicate that CL-1182C and 48/80 produce their hypotensive response by the same mechanism.

CL-1182C increases plasma histamine. The results of four experiments are summarized in Table IV. It is interesting that the histamine levels were highest at the 1 min. determination and decreased significantly within 10 min. There is no significant difference between the 10- and 20-min. histamine levels.

TABLE II.—EFFECT OF CL-1182C ON MEAN ARTERIAL PRESSURE AND PERIPHERAL RESISTANCE IN ANESTHETIZED DOGS

	CL-1182C Dose, P.O.	—Max. Decrease, mm. Hg— Mean Arterial Pressure	Mean Peripheral Pressure
	100 mg./Kg.		
Dog	*1*	115	140
Dog	*2*	120	130
	50 mg./Kg.		
Dog	*1*	No effect	No effect
Dog	*2*	55	65
Dog	*3*	80	75

TABLE III.—EFFECT OF CL-1182C ON SYSTOLIC PRESSURE IN NONANESTHETIZED DOGS AFTER 100 mg./Kg. P.O.

Dog	Treatment	Systolic Pressure, mm. Hg Pre-treatment	Post-treatment
1	CL-1182C	120	70
	Placebo	140	130
2	CL-1182C	190	130
	Placebo	190	190
3	CL-1182C	140	100
	Placebo	140	150
4	CL-1182C	160	110
	Placebo	150	150

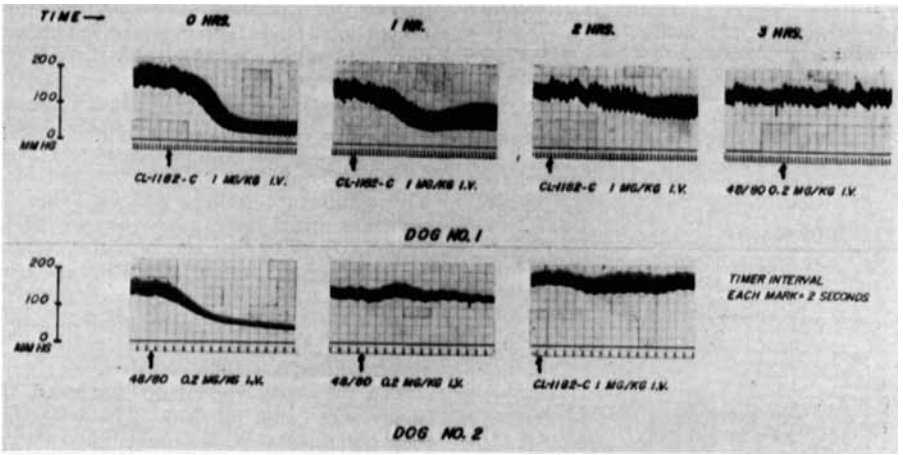


Fig. 3.—Cross tachyphylaxis for CL-1182C and 48/80.

TABLE IV.—INCREASE IN PLASMA HISTAMINE LEVELS OVER CONTROLS CAUSED BY INTRAVENOUS ADMINISTRATION OF CL-1182C

Dose, mg./Kg.	Time After Dosage, min.	Plasma Histamine Increase, mcg./ml. Mean	S.E.	*p*
1.0	1	0.091	±0.0156	<0.05
	10	0.017	±0.0093	>0.10
	20	0.011	±0.0092	

CL-1182C is one of the first compounds of relatively low molecular weight found to be a potent histamine releaser. By comparison on the dog blood pressure, 48/80 is about 200 times as potent a histamine releaser as is polymyxin-B (4). By the same comparison CL-1182C appears to be about one-fifth as potent as 48/80.

Even though CL-1182C has a spasmolytic activity on the isolated ileum, the spasmogenic effect on the gastrointestinal tract caused by histamine release is the predominant effect *in vivo*. The result was violent propulsive defecation.

Being a convulsant, CL-1182C apparently enters the central nervous system. Experiments are now in progress to determine if this convulsant activity is associated with histamine release.

CL-1182C produces histamine release rapidly. After intragastric administration to nonanesthetized dogs, a fall in systolic pressure, salivation, and vomiting occurred as rapidly as 10 min. Plasma levels of histamine were increased tremendously within 1 min. after intravenous administration of CL-1182C. This effect coincides temporally with the vasodepressor response of the compound.

Injection of CL-1182C into mice, rats, and guinea pigs did not cause signs of histamine release. Histamine levels were not determined in the plasma of these animals. The indications are that the potent histamine release effect of CL-1182C is manifested particularly in dogs. Therefore, it differs from 48/80 and polymyxin-B, which produce their effect in other species as well (4, 5).

SUMMARY

CL-1182C, a spasmolytic on the isolated ileum, produces convulsions, vomiting, salivation, and violent propulsive defecation in nonanesthetized dogs. The compound lowers arterial pressure in both anesthetized and nonanesthetized dogs. This hypotension is associated with a fall in peripheral resistance.

The hypotensive responses to CL-1182C and 48/80 are cross tachyphylactic. CL-1182C increases plasma histamine levels after intravenous injection.

The experiments indicate that CL-1182C is a potent histamine releaser in dogs.

REFERENCES

(1) Dragstedt, C. A., *Physiol. Rev.*, **21**, 563(1932).
(2) Paton, W. D. M., *Brit. J. Pharmacol.*, **6**, 499(1951).
(3) Bushby, S. R., and Green, A. F., *ibid.*, **10**, 215(1955).
(4) Paton, W. D. M., *Pharmacol. Rev.*, **9**, 269(1957).
(5) West, G. B., *Clin. Pharmacol. Therap.*, **4**, 749(1963).
(6) Burn, J. H., "Practical Pharmacology," Blackwell Scientific Publications, Oxford, England, 1952, p. 17.
(7) Shore, P. A., Burkhalter, A., and Cohn, V., Jr., *J. Pharmacol. Exptl. Therap.*, **127**, 182(1959).