Brit. J. Pharmacol. (1950), **5**, 125.

THE PHARMACOLOGY OF PHENADOXONE OR *dl*-6-MORPHOLINO-4:4-DIPHENYL-HEPTAN-3-ONE HYDROCHLORIDE

BY

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(Received November 26, 1949)

Since the isolation of morphine by Sertürner in 1816 the search for satisfactory substitutes has been long and intensive. Though morphine is capable of relieving severe pain, its use may involve undesirable side effects, especially respiratory depression and addiction. Attempts have been made so to modify the morphine molecule as to retain the analgesic activity without these side effects (Small, Eddy, Mosettig, and Himmelsbach, 1938). Some success was achieved with the introduction of methyl-dihydromorphinone ("Metapon"; Lee, 1942), but it still had addictive properties. Many compounds unrelated to morphine have also been prepared in an attempt to obtain a synthetic substitute for it, but the first true synthetic analgesic, now known as pethidine (Eisleb and Schauman, 1939), was in fact developed as a spasmolytic. This compound was also found to cause addiction (Polonio, 1947). Many related compounds have since been prepared and tested (Eisleb, 1941; Schauman, 1940; Bergel, Morrison, and Rinderknecht, 1944; Macdonald, Woolfe, Bergel, Morrison, and Rinderknecht, 1946), but only β-pethidine (Bergel, Hindley, Morrison, and Rinderknecht, 1944; Glazebrook and Branwood, 1945) and possibly ketobemidone or Hoechst No. 10720 (United States Department of Commerce, 1946; Kirchoff, 1948) appear to be of importance.

During the World War II German chemists discovered a new group of compounds with analgesic activity. Their work was disclosed in reports published by the United States Department of Commerce (1946) and the British Intelligence Objective Subcommittee (1946). Several of these compounds were very powerful analgesics, especially *dl*-6-dimethylamino-4:4-diphenylheptan-3-one hydrochloride (Hoechst 10820, Amidone or Dolophine) and *dl*-6-morpholino-4:4-diphenylhexan-3-one hydrochloride (Hoechst 10581). Attention has been directed chiefly to the first of these by numerous publications on its pharmacological and clinical properties (Scott and Chen, 1946; Scott, Robbins, and Chen, 1946; Eddy, 1947; Hewer and Keele, 1947 and 1948; Isbell, Wikler, Eddy, Wilson, and Moran, 1947 and 1948; Scott, Kohlstaedt, and Chen, 1947; Thorp, Walton, and Ofner, 1947a, 1947b; Finnegan, Haag, Larson, and Dreyfuss, 1948; Troxil, 1948, etc.).

Scott and his co-workers have also recorded briefly some of the pharmacological properties of Hoechst 10581 (Scott, Robbins, and Chen, 1946 ; Scott, Kohlstaedt, and Chen, 1947). On rats amidone has been shown by Thorp *et al.* (1947a) to have five to ten times the analgesic activity of pethidine and later (1947b) to be 1.3 times as active as morphine. Scott and Chen (1946) found it to be more active on dogs than morphine. Similar results have been found in man by Hewer and Keele (1948). There have been no reports on the analgesic activity of Hoechst 10581 compared with pethidine or morphine, but the threshold doses found by Scott, Robbins, and Chen (1946) and by Scott, Kohlstaedt, and Chen (1947) suggest that compared with amidone it is only one-eighth as active in man, though it is approximately half as active in the rat and dog. The evidence would also suggest that Hoechst 10581 is approximately four times as active as pethidine on the rat and dog, but only twice as active on man.

Holding that amidone in relation to its analgesic activity is unduly toxic, and that a substance with a higher margin of safety would be useful, we have examined a group of related compounds made in an attempt both to decrease the toxicity and to maintain or increase the analgesic activity. The chemistry of these compounds, together with their toxicities and analgesic activities, has already been reported by Dupré, Elks, Hems, Speyer, and Evans (1949) and Attenburrow, Elks, Hems, and Speyer (1949).

We find that the compound *dl*-6-morpholino-4 : 4-diphenyl-heptan-3-one hydrochloride (which differs from Hoechst 10581 in replacement of the hexane by the heptane chain) possesses a higher analgesic activity on rats than any other compound of this type hitherto described, and we have therefore investigated its pharmacocological properties in some detail. This compound was known for laboratory purposes as CB11, and we shall here refer to it in that way for brevity. It is now available for use in the U.K. as a "Schedule IV Poison" under the proprietary name of "Heptalgin." The non-proprietary name Phenadoxone has been approved for it.

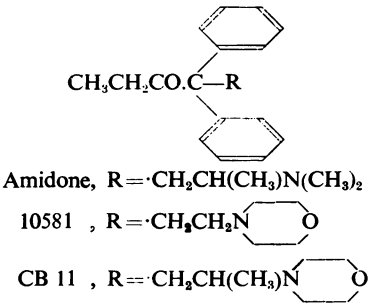
Clinical trials with CB11 have been reported by Wilson and Hunter (1948) and Hewer, Keele, Keele, and Nathan (1949). Their results show that for relieving certain types of pain in human subjects it is a potent analgesic that compares favourably with amidone. Hewer and Keele (1948) also tested it in man and found it to be at least as active as morphine or amidone, with fewer side effects than either of these. Variations in the analgesic responses of different patients were, however, enormous.

We report here some of the pharmacological properties of CB11, compared with those of morphine, pethidine, amidone, and Hoechst 10581 (the hexane analogue of CB11).

During the preparation of our manuscript for publication, brief reports on the pharmacology of this compound have appeared. Speeten, Byrd, Cheney, and Binkley (1949) reported that the compound had an analgesic activity in animals at least as great as that of amidone and yet was less toxic. They also referred to a German claim (P.B. 1-70056. Frames 1229-1232 B) that the compound was a potent analgesic. Flataker and Winter (1949) found it to be twice as active as amidone on rats, but only half as active on dogs. Its acute toxicity to mice by the subcutaneous route was much lower than that of amidone.

Chemical and physical properties

The chemical relationship of CB11 to amidone and Hoechst 10581 is shown below.



CB11 hydrochloride is a colourless, odourless crystalline solid. It is freely soluble in water at *p*H 4; above *p*H 4 the free base begins to come out of its solution, precipitation being complete in weakly alkaline solutions. It can be autoclaved without decomposition in acid solution at *p*H 2.5, but at *p*H 3.5 precipitation may occur unless the solution is suitably buffered. The hydrochloride was used throughout our investigations.

RESULTS

Acute toxicity

The acute toxicity of CB11 was ascertained on albino mice of both sexes descended by rigorous inbreeding from Strong A2 ancestors. Injections were made on a weight basis into animals, weighing between 18 and 22 g., which had fasted for the previous 24 hours. For all routes of administration the regression of mortality per cent, as probits, on log dose was found and graphical estimates of the LD50 and limits of error (P=0.95) were made by the method of de Beer (1945). Table I shows the acute toxicity of CB11 compared with

TABLE I

ACUTE TOXICITIES IN MICE

Limits (P = 0.95), calculated by the graphical method of de Beer (1945), are given in parentheses.

Compound	Molecular weight	LD50 for mice mg./kg.		
		Oral	Subcutaneous	Intravenous
Morphine hydrochloride	375	580 (510–661)	490 (402–598)	196 (174–223)
Pethidine hydrochloride	284	302 (248–365)	202 (184–222)	44 (40–48)
Amidone hydrochloride	346	90 (68–119)	48 (41–57)	19 (17–21)
10581 hydrochloride	374	225 (207–245)	206 (181–233)	30 (27–33)
CB11 hydrochloride	388	208 (177–243)	194 (175–215)	43 (39–47)

those of pethidine, morphine, amidone, and 10581 by the subcutaneous, intravenous, and oral routes. Weight for weight, CB11 is less toxic to mice than amidone. The toxicities of CB11 and compound 10581 are of the same order by all routes. Our LD50 values for amidone, pethidine, and 10581 by the intravenous route are in close agreement with those reported by Scott, Kohlstaedt, and Chen (1947). Toxic doses in mice produce a syndrome similar to that seen after the administration of morphine. At first there is a potentiation of movement and an erection of the tail (Straub-Hermann reaction); this is followed by tetany, coma, and death, which is primarily due to respiratory depression.

Analgesic activity

The analgesic activity of CB11 was determined by the subcutaneous route in rats using (*a*) the thermal radiation ("rat tail") method (Thorp, 1946) and (*b*) the electric grid ("rat grid") method (Dodds, Lawson, Simpson, and Williams, 1945).

(*a*) *Thermal radiation method.*—The method depends upon the rat's reaction to a heat stimulus applied to a small area of its tail and is based on the method of D'amour and Smith (1941): we used the apparatus of Hardy, Wolff, and Goodell (1940) as modified by Thorp (1946); our general technique was essentially similar to his. The individual pain threshold values were measured for each rat as the voltage required on the lamp to elicit a response by the end of the constant time (7½ seconds) during which the shutter was opened. The drugs were injected subcutaneously, on a body-weight basis, at two-minute intervals. The subsequent rise in the pain threshold was measured for each rat exactly thirty minutes after the injections, preliminary experiments having indicated that this was the time of peak analgesia with CB11. Pure line albino rats of Wistar strain origin in the weight range 40 to 60 grammes were used throughout and were randomly distributed into groups. All solutions were prepared so that the doses were present in a volume of 0.4 ml. per 100 g. rat. The mean percentage rise in the pain threshold over the initial was determined for each group of rats and a linear regression relating this to log dose was calculated.

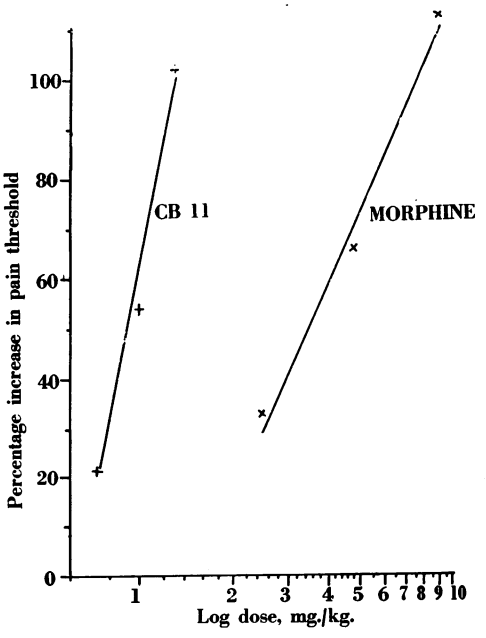


FIG. 1.—A comparison between the analgesic effects of CB11 and morphine in rats. (Radiant heat method.)

In the first experiment we compared the analgesic activity of CB11 with that of morphine hydrochloride B.P. Groups of five rats were used at each dose level, and the comparison was repeated three times on three alternate

TABLE II

THE COMPARATIVE ANALGESIC ACTIVITIES OF CB11 AND MORPHINE IN RATS BY THE RADIANT HEAT METHOD

Drug	No. of rats	Dose: mg./kg.	Mean rise in pain threshold %	Fiducial limits (P = 0.95)	Slope† "*b*"	Dose to give 50% rise: mg./kg.	Approximate activity ratios* Morphine=1
Morphine HCl	15 15 15	2.5 5.0 10.0	33 66 113	±12.1	133	3.67 Limits (P=0.95): 3.14 to 4.17	1.0
CB11 ..	15 15 15	0.75 1.00 1.33	21 54 102	±14.1	242	0.96 Limits (P=0.95): 0.88 to 1.04	3.8

* Ratios calculated at 50 per cent level. † "*b*" is measured in units of percentage increase in the pain threshold per tenfold increase in the dose.

days. The results obtained are shown in Table II and Fig. 1 and the relevant analysis of variance in Table III. This analysis shows that a significant regression had been established for both drugs, but that there was a significant departure from parallelism, so that estimates of the relative analgesic activities of the two compounds cannot be valid over a range of doses. Equiactive doses of morphine and CB11 producing a 50 per cent rise in the pain threshold are given in Table II, column 7, and it is on this basis that approximate activities are quoted here.

A comparison was then made of the analgesic activities of CB11 with amidone and Hoechst 10581, a group of thirty rats being used on each of four days, as recently described by Thorp (1949). Two dose levels were employed for each compound. The rats were changed over daily so that each group of rats received each compound at one of the two dose levels. The doses were administered, as before, subcutaneously at two-minute intervals. Table IV shows the results obtained. The slopes again show a significant departure from parallelism, the

TABLE III

ANALYSIS OF RESULTS IN TABLE II

	Morphine				CB11		
	Degrees of freedom	Mean square	Variance ratio	P	Mean square	Variance ratio	P
Occasion of test ..	2	0.422	0.09	Not significant	40.2	5.48	0.05–0.01
Linear regression on dose	1	480	89.3	Significant	488	66.5	Significant
Deviation from linearity	1	5.378	1.0	Not significant	4.9	0.67	Not significant
Error	40	5.372					

TABLE IV

THE COMPARATIVE ANALGESIC ACTIVITIES OF AMIDONE, HOECHST 10581, AND CB11 IN RATS BY THE RADIANT HEAT METHOD

Drug	No. of rats	Dose: mg./kg.	Mean rise in pain threshold* %	Slope	Dose to give 50% rise: mg./kg.	Approximate activity ratios (amidone=1)
Amidone..	20	1.8	27.6	121.8	2.6 Limits (P=0.95): 2.24 to 3.12	1.0
	20	3.6	64.3			
Hoechst 10581	20	2.25	23.3	95.0	4.0 Limits (P=0.95): 3.34 to 6.07	0.65
	20	4.5	51.9			
CB11 ..	20	0.9	48.1	196.1	0.9 Limits (P=0.95): 0.77 to 1.0	2.9
	20	1.8	110.0			

* These values have fiducial limits (P=0.95) of ±19.4 per cent calculated on all the data.

one for CB11 being steeper than those for the other two drugs (Fig. 2). The activity ratios, which are quoted in terms of amidone hydrochloride, can therefore only apply at a particular response level, the ones quoted being those found for a 50 per cent rise in the pain threshold.

(*b*) *Electric grid method.*—The previous method determines the depth of analgesia thirty minutes after the injections, no regard being paid to duration. We therefore compared CB11 with morphine and other drugs, after subcutaneous injection in rats, by the electric grid method of Dodds, Lawson, Simpson, and Williams (1945), which measures the tolerance of rats to electric shocks. The apparatus used was similar to theirs, but we varied the alternating current in the primary coil of the Du-Bois–Raymond apparatus, using a variable rheostat having a scale in arbitrary units calibrated in proportion to voltage. In all tests the current was increased until the rat squeaked, the rheostat reading being then recorded. Determinations of the pain thresholds for individual rats were made before the injections and at 20-minute intervals during two hours.

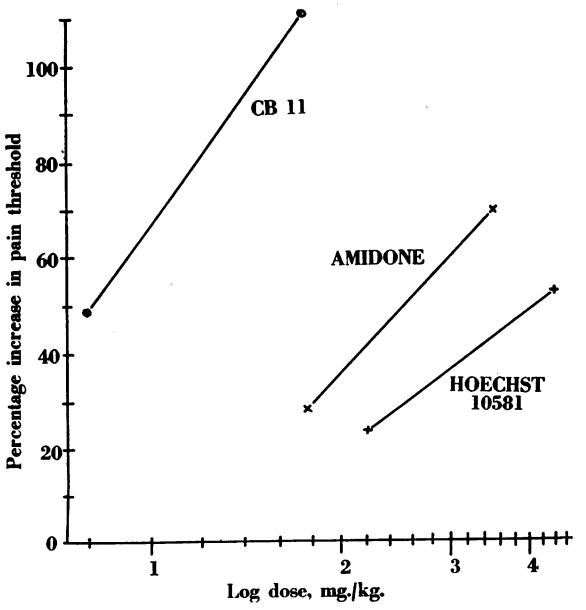


FIG. 2.—A comparison between the analgesic effects of CB11, amidone, and Hoechst 10581 in rats. (Radiant heat method.)

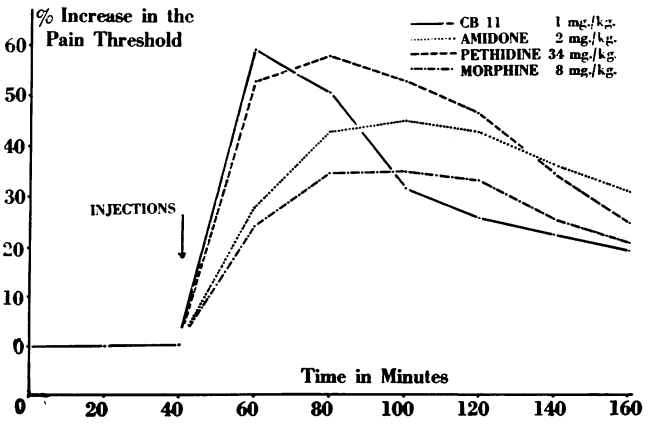


FIG. 3.—A comparison between the analgesic effects of CB11, amidone, pethidine, and morphine in rats. (Electric grid method.)

Fig. 3 shows the results obtained, each point being for the mean observation from eight rats. The onset of analgesia with CB11 is extremely rapid, optimal activity being apparent within 20 minutes after injection, and the drug gives a big rise in the pain threshold. Amidone is quick acting; the onset of analgesia with morphine and pethidine is slower. Both Thorp (1946) and Dodds and his co-workers (1945) recorded similar findings for morphine, and the latter also for pethidine. The duration of analgesia with CB11 is shorter than with the other drugs. Assessment of the relative analgesic activities of these compounds will obviously vary with the time interval between the injection and the measurement of the rise in pain threshold. Both methods indicate that CB11 is a powerful analgesic for the rat, being by the subcutaneous route more active than morphine or amidone on a weight basis.

The differences in slope of the regression lines for the various compounds makes a stricter quantitative comparison meaningless. It is only to be expected that compounds with diverse chemical structures will vary in both the mode and the duration of action, and for that reason alone it would seem illogical to quote their activities in terms of each other. Further, it is unlikely that ratios so determined on rats would apply to other species, including man.

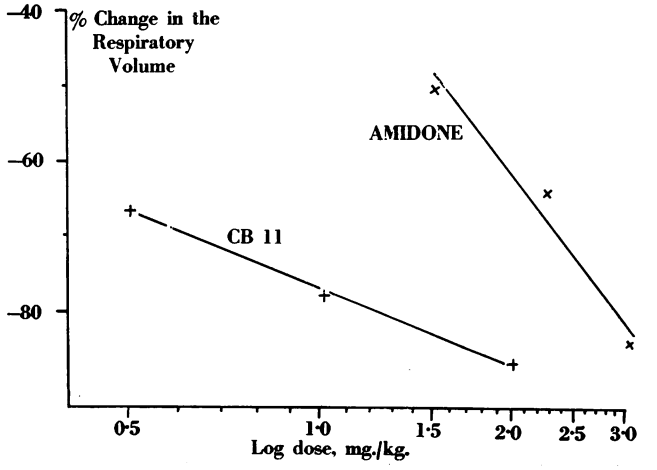


FIG. 4.—The effect of CB11 and amidone on the respiratory minute volume in rabbits.

Respiratory depression

Respiratory depression was studied in conscious rabbits with a recorder, of the differential type described by Gaddum (1941), connected to a rubber mask fitted with unidirectional valves. The rabbits were restrained in special boxes

TABLE V

THE EFFECT OF CB11 AND AMIDONE ON THE RESPIRATORY MINUTE VOLUME IN RABBITS

CB11			Amidone		
Dose: mg./kg.	Maximum change in respiration %		Dose: mg./kg.	Maximum change in respiration %	
	Recorded	Mean		Recorded	Mean
0.5	−70 −74 −32 −75 −74 −57	−64	1.5	−36 −54 −35 −62 −55	−48
1.0	−69 −95 −78 −90 −45	−75	2.25	−63 −63 −61 −60 −59	−61
2.0	−76 −93 −80 −88 −84	−84	3.0	−84 −85 −80 −81 −75	−81

(" stocks ") and kept warm throughout the experimental period. The compounds were tested at three dose levels, five to six rabbits being used for each dose. A direct comparison of CB11 was made with amidone, subcutaneous injections being made on a body-weight basis. The apparatus recorded a response linearly proportional to the respiratory minute volume. The peak percentage of respiratory depression was measured, and the results (shown in Table V) give the maximum respiratory depression as a percentage of the normal respiratory minute volume. CB11 is a stronger respiratory depressant than amidone (Fig. 4). A 75 per cent depression would be produced by approximately 1.0 mg./kg. of CB11 or 2.75 mg./kg. of amidone, giving a ratio of 2.75 to 1.0, which is related to the relative analgesic activities of the two compounds. Thorp (1947 and 1949) has shown that respiratory depressant action parallels analgesic activity with amidone and related compounds. Intravenously, 0.3 mg./kg. of CB11 caused a 90 per cent respiratory depression in sixteen rabbits.

In six cats under chloralose 0.2 mg./kg. of CB11, injected intravenously, caused a slight respiratory depression, while 0.5 mg./kg. had a strong effect. The records, obtained by the apparatus of Wilbrand and de la Cuadra (1947) connected to the side arm of the tracheal cannula, show that CB11 had a stronger respiratory depressant action than amidone (Fig. 5) or Hoechst 10581 ; 1.0 mg./kg. frequently arrested respiration completely.

CB11, like amidone, in excessive doses can cause a profound respiratory depression, which is the primary cause of death after toxic doses. Respiratory depression is particularly marked by the intravenous route. Providing the respiration is maintained, however, the animal will usually survive.

The analeptic drugs nikethamide and picrotoxin, especially the former, are of value in reversing this effect of these drugs. The respiration of cats under chloralose, depressed by the intravenous administration of 0.5 mg./kg. of CB11, was increased by a dose of 8 mg. nikethamide per kg. injected intravenously. At the same time the blood pressure was restored (Fig. 6). Picrotoxin was only partially effective at a dose level of 0.7 mg./kg., and repeated doses were necessary to stimulate respiration. Amphetamine was practically ineffective in doses up to 0.33 mg./kg.

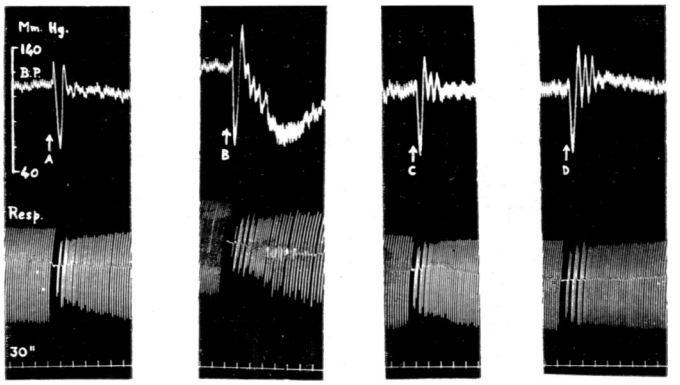


FIG. 5.—Effects of intravenous injections of CB11 and amidone in a cat anaesthetized with chloralose (0.08 g./kg.). Upper tracing, blood pressure; lower tracing, respiration. (A) 0.2 mg./kg. of CB11. (B) 0.5 mg./kg. of CB11. (C) 0.2 mg./kg. of amidone. (D) 0.5 mg./kg. of amidone.

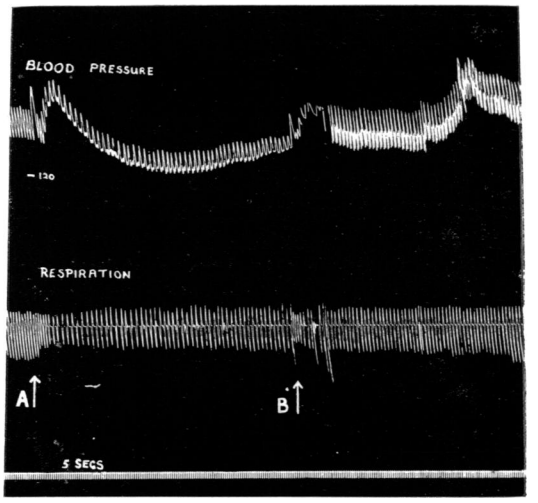


FIG. 6.—Stimulant action of nikethamide on the respiration of a cat after a high intravenous injection of CB11. (A) 0.5 mg./kg. of CB11. (B) 8.0 mg./kg. of nikethamide.

Heart

Effects on the isolated rabbit heart.—We first studied the cardiac effects of CB11 on the isolated heart of the rabbit, perfused from the aorta through the coronary vessels with oxygenated Ringer-Locke solution (Langendorff preparation). The drug was administered (*a*) mixed with the perfusion fluid in a concentration of 1:500,000 or 1:100,000 and (*b*) by direct injection into the cannula. Records were obtained of both the rate and the amplitude of the beat together with the coronary flow.

Results obtained on perfusing the hearts with solutions containing CB11 or amidone are shown in Table VI. Their actions were closely similar, the toxic level for both drugs being a concentration of 1:100,000 or greater. Our results for amidone agree with Thorp's (1949). Perfusion again with normal Ringer solution restored the hearts, but arrhythmias, which frequently developed, were

TABLE VI

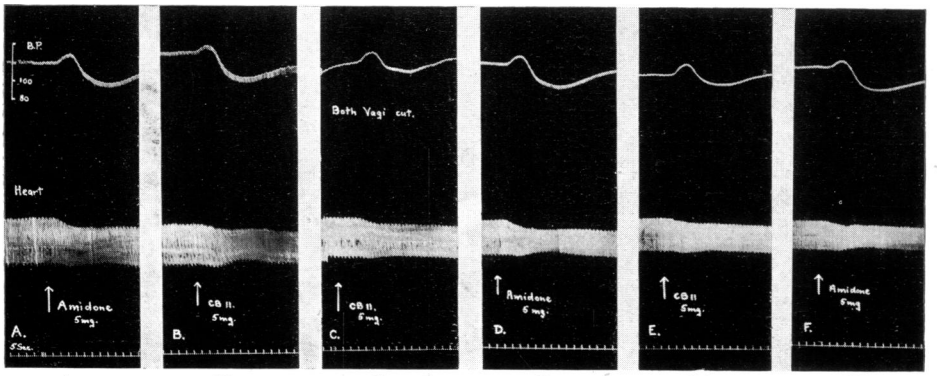
THE EFFECT OF CB11 AND AMIDONE UPON THE ISOLATED RABBIT HEART (LANGENDORFF PREPARATION)

Drug	Dilution	Effect upon isolated heart		
		Coronary flow	Heart rate	Amplitude
Amidone	1 : 500,000 1 : 100,000	+11% +103%	Nil Slowed	Slightly decreased Decreased
CB11	1 : 500,000 1 : 100,000	+49% +144%	Nil Slowed	Nil Decreased

not abolished. The most striking effect of the compounds was on the amplitude of the heart beat, the heart rate being only slightly diminished. Both drugs increased coronary flow.

Direct injection of CB11 or amidone (0.025 and 0.05 mg.) into the cannula reduced the amplitude of the contractions, but only slightly reduced the rate. Atropine did not prevent the cardiac depressant action.

Effects on the heart in situ.—The actions of CB11 and amidone on the hearts of six cats, artificially respired and under pentobarbitone anaesthesia, were studied *in situ*. A Cushny myocardiograph was attached to the ventricles. Doses of 2 mg. CB11 per kg. injected rapidly into the femoral vein reduced the amplitude of the heart beat, but had very little effect on the rate. Recovery was rapid. Assuming the blood volume to be 6.5 per cent of the bodyweight, the concentrations of the drug in the blood would be similar to those depressing the isolated heart. The blood pressure showed a concomitant fall. The cardiac effects were not abolished by cutting the vagi or after atropine (Fig. 7). Both amidone and Hoechst 10581 at this dose level had effects similar to CB11.



A B C D E F

FIG. 7.—Effects of intravenous injections of CB11 and amidone in a cat (2.5 kg.) anaesthetized with pentobarbitone. Upper tracing, blood pressure; lower tracing, ventricular contractions. (A) 2 mg./kg. of amidone. (B) 2 mg./kg. of CB11. (C) 2 mg./kg. of CB11 after section of the vagi. (D) 2 mg./kg. of amidone after section of the vagi. (E) 2 mg./kg. of CB11 after atropine. (F) 2 mg./kg. of amidone after atropine.

Circulatory effects

The circulatory effects of CB11 and amidone were compared in fifteen cats, anaesthetized with chloralose or pentobarbitone. The blood pressure was recorded from the carotid artery on a mercury manometer in the usual manner; injections were made into the femoral vein. In some tests simultaneous records were obtained of the respiration, using the apparatus of Wilbrand and de la Cuadra (1947) or from a thread attached to the ribs. Initially the blood pressure in the naturally breathing animal showed an immediate fall equivalent to about 35 mm. of mercury after a first injection of 0.2 mg./kg. The fall was associated with a reduced heart rate. Recovery was rapid and the blood pressure had returned to normal one minute after the injection. Respiration was completely arrested for 30 seconds and then recommenced at a lower depth (Fig. 5). Recovery was usually complete within five minutes. With 0.5 mg./kg. the same abrupt fall in blood pressure occurred, followed by a rapid recovery, but this was in turn followed by a slower and longer fall associated with respiratory depression (Fig. 5). In artificially respired cats doses as high as 1.0 mg./kg. do not cause a blood pressure fall (Fig. 8). Doses of 2.0 mg./kg. are necessary to depress the blood pressure (Fig. 7). the drug having a slight cardiotoxic action at this dose level. A rapid intravenous dose of 5.0 mg./kg. in the unrespired preparation would be fatal, the respiration becoming completely arrested. Amidone affected the blood pressure similarly (Fig. 7), as Thorp (1949) found.

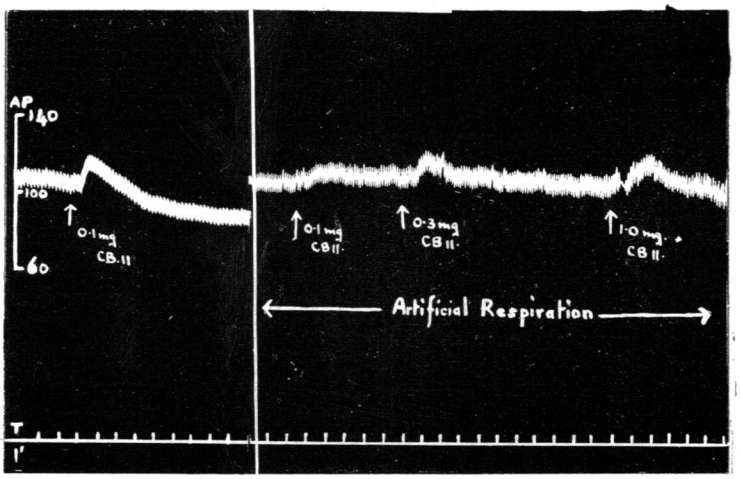


FIG. 8.—Effects of intravenous injections of CB11 on the blood pressure of a cat anaesthetized with chloralose (0.08 g./kg.). (A) 0.1 mg./kg. of CB11. (B) 0.1 mg., (C) 0.3 mg., and (D) 1.0 mg. per kg. of CB11 during artificial respiration.

With repeated doses of 0.2 mg./kg. of CB11 at 30-minute intervals the abrupt fall in blood pressure diminished, indicative of "acute vascular tolerance," which has been reported by Shideman and Johnson (1947) to occur with amidone and is common to most analgesic drugs.

On the blood vessels themselves CB11 had very little effect. The vessels of the hind legs of three cats were perfused with heparinized blood by means of a Dale-Schuster pump (Dale and Schuster, 1928); records were obtained of the perfusion pressure and of the venous outflow by means of a Gaddum Drop

Recorder. A slight vasodilator effect occurred when 1.0 mg. CB11 was injected directly into the inflowing perfusion fluid. Amidone and Hoechst 10581 had the same effect at this dose level. The tone of the vessels remained unaffected and the response to adrenaline unchanged.

Central nervous system

In artificially respired cats under chloralose it has been constantly observed that large doses of both CB11 and amidone produce an excitation of the central nervous system in the form of severe clonic convulsions. The convulsions that occur after an intravenous injection of 1 mg./kg. of CB11 can be abolished by the adminstration of a barbiturate and do not occur in spinal cats. In high doses the drug appears to be a central stimulant in this species. Scott and Chen (1946) and Wikler, Frank, and Eisenman (1947) have reported similar effects with amidone in cats.

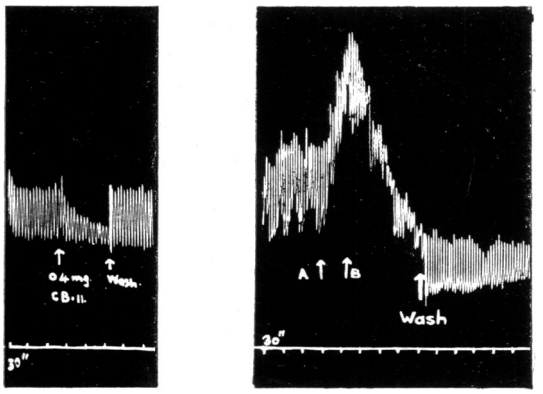
Actions on smooth muscle

Isolated intestinal muscle.—The actions of CB11 were studied on isolated rabbit duodenum and guinea-pig ileum segments suspended in a bath of oxygenated Ringer solution at 37° C.

In the rabbit duodenum the normal rhythmic contractions were reduced by 0.4 mg. CB11 added to a 40 ml. bath (Fig. 9). Recovery occurred a few minutes after washing out. Amidone, pethidine, morphine, and 10581 had similar effects, but with pethidine they were quicker in onset and of shorter duration.

CB11 had a weak spasmolytic action in this preparation, the spasms produced by 1 μg. acetylcholine or 50 μg. barium chloride being relaxed after the addition of 0.4 mg. CB11 to the bath (Fig. 9).

In the guinea-pig ileum, the spasmolytic action of CB11 was more fully investigated by the method of Lee, Dinwiddie, and Chen (1947), in which is measured the degree of relaxation produced by different doses of the spasmolytic agents against an induced spasm of the isolated intestine, produced by histamine, acetylcholine, or barium chloride. The spasmolytic activities of CB11, amidone, and pethidine so determined are shown in Table VII. These are given as equiactive doses producing a 50 per cent relaxation of the spasm. The spasmolytic activity shows no relationship to analgesic activity. The power of CB11 to antagonize histamine, determined by the method described by Schild (1947), gave a value for pA_2 of 6.4: for pethidine we found 6.3.



(*a*) (*b*)

FIG. 9.—(*a*) Effect of CB11 on the isolated rabbit duodenum (0.4 mg. in 40 ml. bath). (*b*) Antispasmodic action of CB11 on the rabbit duodenum. (A) Spasm produced by 0.5 ml. of barium chloride (1 in 10,000). (B) 0.4 mg. of CB11.

TABLE VII

THE SPASMOLYTIC ACTIVITIES OF CB11, AMIDONE, AND PETHIDINE ON THE ISOLATED GUINEA-PIG ILEUM

Drug	Dose in mg. in 40 ml. bath required to cause 50% relaxation of the spasm produced by		
	1 μg. acetylcholine	20 μg. histamine	60 μg. $BaCl_2$
CB11	0.04	0.02	1.0
Amidone	0.07	0.02	1.0
Pethidine	0.05	0.05	2.0

Intestine in situ.—Rabbits were lightly anaesthetized with urethane, and the abdomens opened; the peristaltic movements of a 3 cm. length of the jejunum were recorded by means of an attached thread. The abdominal cavity was filled with warmed Ringer's solution maintained at 37° C. Depression of peristalsis only occurred after the intravenous injection of very high doses of CB11 (at least 2 mg./kg.) and this caused severe respiratory depression, necessitating the use of artificially respired animals. These findings support our observations in rats that the drug has practically no constipating action.

Action on the uterus.—On isolated virgin guinea-pig uteri, suspended in Ringer's solution, CB11 abolished any spontaneous contractions present at a dose level 0.05 mg. CB11 in a 40 ml. bath. This dose also reduced the amplitude of the contractions to submaximal doses of posterior pituitary extract. The action is possibly related to the spasmolytic action of the drug on smooth muscle.

Local anaesthetic activity

Everett (1948) reported that amidone had a local anaesthetic action on the rabbit's cornea and also on the human intradermal weal. We have examined CB11 for this property. When tested on the guinea-pig corneal reflex, by the method of Chance and Lobstein (1944), CB11 had some local anaesthetic action. The ED50 values for procaine hydrochloride and CB11 were 21 mg./ml. and 13.4 mg./ml. respectively. CB11 is therefore more active than procaine in producing surface anaesthesia. The onset of local anaesthesia was slower than with procaine hydrochloride.

By the intradermal weal method in guinea-pigs (Bülbring and Wajda, 1945) CB11 had only a slight activity, solutions containing 20 mg. per ml. producing an anaesthetic action for only ten minutes. Procaine hydrochloride at a concentration of 1.25 mg./ml. showed a high activity by this method. The local anaesthetic action of CB11 is thus too low to be of any practical importance.

Metabolic effects

Large doses of CB11, as of morphine and amidone, cause a fall in the body temperature of rabbits, but in therapeutic doses the depression is not significant.

Chronic toxicity

Rats.—Twenty rats received daily subcutaneous injections of 1.0 mg./kg. of CB11, and further groups 2.0 mg./kg. of CB11 subcutaneously and 4.0 mg./kg. orally. Treatment of all animals was continued for over three weeks. At three-day intervals they were tested for analgesia by the radiant heat method, and the doses were proved to be analgesic. Daily records of food consumption and body-weight were compared with those for untreated controls. The treatment caused only slight retardation of growth and no loss of appetite. Samples of urine collected from time to time showed no abnormalities.

Autopsy of the animals at the end of the experiment revealed no gross pathological changes. Histological examinations of the liver, kidneys, spleen, heart muscle, suprarenals, and thyroids showed no abnormalities. Frozen sections of the livers and kidneys, stained for fat, showed no fatty degeneration in any of the animals.

Rabbits.—CB11 was administered daily by stomach tube to two rabbits for 21 days, at a dose level of 0.5 mg./kg. A further four rabbits were injected subcutaneously, two receiving 0.3 mg./kg. daily and two 1.3 mg./kg. daily, all for 21 days. The rabbits continued to grow and maintained their appetites. Blood samples, taken twice weekly, showed no significant changes in the red, white, and differential cell counts or in the haemoglobin concentrations. The drug did not affect the blood sugar levels of fasted rabbits. The urine was examined daily and no abnormal constituent was found. At autopsy there was no macroscopic evidence of gastric or intestinal irritation in the orally dosed rabbits or at the sites of injection in the injected ones. Histological examination of sections of the major organs showed no abnormalities and frozen sections of the livers and kidneys showed no fatty degeneration.

Urinary excretion

Antidiuretic effect.—CB11 had a slight antidiuretic action at high dose levels. Groups of four male rats, weighing approximately 150 g., were given, by stomach tube, 5 ml. of warm water per 100 g. bodyweight. CB11 was injected subcutaneously, and the urine excreted during the next six hours was collected. Two groups received 2.5 mg. and 1.25 mg./kg. of CB11, a third group saline solution only. The test was repeated three times, the groups being crossed over.

The high dose of CB11 reduced urine excretion to 30 per cent below that of the controls. The low dose had little effect. The antidiuretic action was only slight and the high doses caused drowsiness in the rats.

Renal excretion.—Attempts to determine the urinary excretion of this drug in rats have so far given extremely variable results. The amount excreted over 24 hours is undoubtedly small and the indications are that it is about 15 per cent. Since the maximum dose of CB11 tolerated by rats is small, urinary concentrations are low, making chemical determinations difficult. Further, the method of assay (Page and King, 1950), although satisfactory for cat and human urine, gave seriously inconsistent results with rat urine, apparently because it yields high and variable blank readings. The method was an adaptation of that described by Lehman and Aitken (1942), but bromophenol blue was used as indicator and

the coloured complex was formed in a phthalate buffer solution at *p*H 4.0. In artificially respired cats, anaesthetized with chloralose and having the bladder cannulated, 30 per cent of the drug was excreted during a period of six hours after an intravenous injection of 5 mg. per kg. Conflicting results have also been reported in using colorimetric methods for determining the urinary excretion of amidone (Scott and Chen, 1946; Cronheim and Ware, 1948). Elliott, Chang, Abdou, and Anderson (1949), using amidone labelled with ^{14}C in the 2 position, have been able to show that in rats practically 100 per cent excretion occurs within 24 hours, approximately 30 per cent in the urine and the rest in the faeces.

Irritant effects

Subcutaneous injection into guinea-pigs of a 1 per cent aqueous solution caused no inflammation or oedema at the site of the injection, providing the *p*H of the solution was not too high. With intramuscular injections there were no untoward reactions, and only slight irritation was caused in instilling a 1 per cent buffered solution of CB11 into the eye. By the intravenous route solutions of CB11 had no sclerosing effect on the veins. Solutions at *p*H 4.5 have never produced necrosis in concentrations up to 1 per cent.

Tolerance

Rats dosed daily with 2 mg./kg. by the subcutaneous route showed no evidence of tolerance. The elevation of the pain threshold, tested at weekly intervals for three weeks, was not diminished.

Pupil size

Neither parenteral nor local administration of solutions of CB11 causes constriction of the pupil in the rabbit.

Discussion

CB11 is one of some forty amino-ketones and amino-esters related to amidone that have been synthesized and examined in these laboratories for analgesic activity and toxicity. Of all compounds examined, CB11 was the most active and its pharmacological properties were therefore investigated in some detail.

It appears that the compound is a very potent analgesic for the rat; by the subcutaneous route it is more active than either morphine or amidone. In spite of this its acute toxicity to mice is lower than that of amidone and its therapeutic index is therefore correspondingly higher, giving a wider margin of safety. These findings confirm those of Flataker and Winter (1949), who showed that side effects in dogs, such as narcosis, sedation, and general depression, were much less with CB11 than with amidone or morphine. Nausea and vomiting did not occur after CB11 in non-tolerant dogs.

In general, its pharmacological properties are closely similar to those of amidone. At therapeutic dose levels undesirable pharmacological effects, such as cardiac depression and vasomotor disturbance, are absent, and it is only at extremely high dose levels that untoward effects occur. However, the drug has a strong respiratory depressant action when given in high doses; it should be used with special caution if injected intravenously.

We have safely administered analgesic doses of CB11 daily to animals over a prolonged period without impairing their health or producing any pathological effects. No irritant actions have been observed after injection by the subcutaneous, intramuscular, and intravenous routes. So far we have not been able to demonstrate the development of tolerance to the drug in rats. Flataker and Winter, however, reported that dogs, dosed twice daily with CB11 (4 mg./kg.), developed analgesic tolerance in about a month.

Preliminary clinical reports (Hewer and Keele, 1948; Wilson and Hunter, 1948; Hewer, Keele, Keele, and Nathan, 1949) have shown that CB11 has a potent analgesic action on human subjects and compares favourably with morphine and amidone. There is evidence that concomitant side effects are less for CB11 than for morphine or amidone (Hewer and Keele, 1948).

Summary

1. A preliminary investigation has been made into the pharmacological properties of a new analgesic compound, phenadoxone or *dl*-6-morpholino-4:4-diphenylheptan-3-one hydrochloride (CB11). It has been shown to be more active on rats than morphine, amidone, pethidine, or Hoechst 10581.

2. In spite of this high analgesic potency, it is less toxic to mice than amidone and compares favourably with morphine, 10581, and pethidine.

3. Cardiac and circulatory effects in cats were slight, but a high dose given intravenously produced a strong depressant action on the respiratory centre.

4. Respiratory depressant activity appeared to be related to the analgesic potency of these compounds.

5. Like pethidine, amidone, and 10581, the drug had a weak spasmolytic action on smooth muscle.

6. Tests for local anaesthetic action showed this to be only slight for CB11, but it has some surface analgesic action.

7. Like morphine, amidone, and 10581, CB11 caused a slight fall in the body temperature of rabbits, and also had a slight antidiuretic action at high dose levels in rats.

8. A solution of the compound at *p*H 4.5 was free from irritant effects on injection and has been safely administered over a prolonged period to rats and rabbits.

9. There was no evidence of tolerance developing in rats within three weeks.

In the experiments recorded above, we have received the technical assistance of Mr. E. A. Woollett and Mr. M. E. Fielding, to whom we wish to extend our thanks.

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