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EXPERIMENTAL OBSERVATIONS ON HAFFNER'S METHOD FOR TESTING ANALGESIC DRUGS

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Haffner's (1929) method for testing analgesic drugs has been neglected since D'Amour and Smith (1941) applied to animals the thermal pain stimulus devised by Hardy, Wolff and Goodell (1940). Certain objections can, however, be raised against the use of a thermal stimulus for testing analgesic activity. Thus the response to a painful stimulus produced by heat in animals (skin-twitch or tail-flick) involves the spinal cord (Cook and Bonnycastle, 1951 ; Houde and Wikler, 1951 ; Irwin, Houde, Bennett, Hendershot and Seevers, 1951 ; Winter and Flataker, 1951 ; Bonnycastle, Cook and Ipsen, 1953 ; Herr, Nyiri and Venulet, 1953), whereas in man pain sensation is mediated through the thalamus and cortex. It would therefore be obviously desirable to test analgesic drugs by a method involving the highest centres. Furthermore, local temperature of the tail or of the skin can modify the reaction to a thermal stimulus: analgesic drugs can decrease local temperature (Jackson, 1952 ; Winter and Flataker, 1953). The apparatus described by D'Amour and Smith (1941) is complex and the results quoted by various authors are conflicting ; indeed, the estimates of activity obtained from quantal or graded responses are not identical (Bonnycastle and Leonard, 1950).

By contrast Haffner's method is simple, and has the advantage that the reflex mechanism on which it is based involves the higher centres. The animal has to identify exactly the place where the noxious stimulus is applied, and it carries out co-ordinated movements to remove it. In order to test the reliability of Haffner's method we have compared the analgesic power of morphine, pethidine and methadone evaluated according to Haffner with the analgesic power of the same drugs evaluated according to methods now commonly used.

METHODS

An artery clip with the branches enclosed in a thin rubber tube is applied to the root of the tail of a mouse for 30 sec.; the animal makes continuous attempts to remove the noxious stimulus by biting the clip. The mice are injected subcutaneously or intraperitoneally with an analgesic drug, and, after 30 min., the artery clip is applied for 30 sec. The results are expressed as the percentage of mice showing analgesia—insensitivity to the noxious stimulus—after a given dose of analgesic. The following drugs were used: morphine hydrochloride, pethidine hydrochloride, racemic methadone hydrochloride, *laevo*methadone bitartrate ($[\alpha]_D^{20} - 79.6$), *dextro*-methadone hydrochloride ($[\alpha]_D^{24} + 125$)—all in physiological saline.

RESULTS

In a first group of experiments we have examined the intensity of analgesic action. Table I shows the results obtained. The doses for all compounds are given as the hydrochlorides. A linear relation (Fig. 1) was observed between the logarithm of the dose and the probit of mice showing analgesia. The ED50 and its fiducial limits were estimated by the method of Litchfield and Wilcoxon (1949). The ED50 for morphine in this series was 5.7 mg./kg. In another group of 100 mice the ED50 of morphine hydrochloride injected subcutaneously was 5.8 mg./kg. (with 19/20 fiducial limits 5.37 and 6.26). This agrees with the result quoted in Table I.

Analgesic action increases significantly when morphine, pethidine, (±)- and (−)-methadone are injected subcutaneously instead of intraperitoneally. The relative potencies of these drugs when given subcutaneously and intraperitoneally are shown below. The figures in parentheses are the 19/20 fiducial limits.

morphine	1.36 (1.06–1.74)
pethidine	1.44 (1.06–1.94)
(±)-methadone	1.76 (1.32–2.34)
(−)-methadone	1.61 (1.15–2.23)

The relative activities of some of these drugs are shown in Table II. It will be seen that (±)-methadone and (−)-methadone are always more active than morphine, whilst pethidine and (+)-methadone are always less active than morphine ; (−)-methadone is more active than (±)-methadone.

In a second group of experiments we studied the time course of analgesia. Four groups of 20

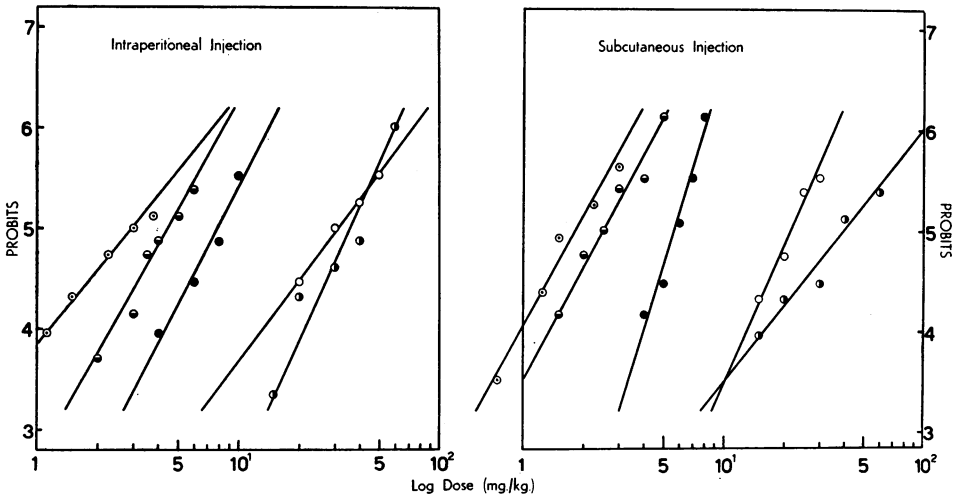

FIG. 1.—Showing the analgesic activity of various drugs when tested by Haffner's method in mice 30 min. after subcutaneous or intraperitoneal injection. Abscissa, log dose in mg./kg. Ordinate, probits. ●, morphine; O, pethidine; ◒, (±)-methadone; ⊙, (−)-methadone; ◑, (+)-methadone.

TABLE I

ANALGESIC ACTIVITY (INTENSITY) IN MICE: ED50 AND 19/20 CONFIDENCE LIMITS

Drug	Route of Administration			
	Subcutaneous		Intraperitoneal	
	ED50 mg./kg.	No. of Mice	ED50 mg./kg.	No. of Mice
Morphine ..	5·7 (4·9–6·6)	65	7·8 (6·4–9·4)	80
Pethidine ..	21·5 (18·6–24·7)	80	31·0 (23·8–40·3)	80
(±)-Methadone	2·5 (2·0–3·0)	75	4·4 (3·6–5·2)	110
(−)- ,,	1·8 (1·4–2·2)	75	2·9 (2·2–3·7)	100
(+)- ,,	40·0 (30·7–52·0)	100	36·0 (30·0–43·2)	100

The doses of all drugs are expressed in terms of the hydrochlorides.

TABLE II

ACTIVITY RATIOS OF ANALGESIC DRUGS BY INTRAPERITONEAL AND SUBCUTANEOUS INJECTION

The figures in parentheses are the 19/20 fiducial limits

Drugs	Route of Administration	
	Intraperitoneal	Subcutaneous
(±)-Methadone/morphine ..	1·77 (1·36–2·30)	2·28 (1·75–2·96)
(−)- ,, ,, ..	2·68 (1·95–3·67)	3·16 (2·46–4·04)
(+)- ,, ,, ..	0·21 (0·16–0·27)	0·14 (0·10–0·18)
Pethidine/morphine ..	0·25 (0·17–0·34)	0·26 (0·21–0·31)
(−)-Methadone/(±)-methadone	1·39 (1·02–1·87)	1·51 (1·10–2·06)

TABLE III

DURATION OF ANALGESIC ACTIVITY. PERCENTAGE OF MICE SHOWING ANALGESIA AFTER RECEIVING DOSES OF ANALGESIC DRUGS CORRESPONDING TO ED80 (SUBCUTANEOUS INJECTION)

	½ Hr.	1 Hr.	2 Hr.	3 Hr.	4 Hr.
Physiological saline	0	0	0	0	0
Morphine ..	75	—	15	0	—
Pethidine ..	70	50	7	5	0
(−)-Methadone ..	75	45	10	5	0

TABLE IV

TOLERANCE TO ANALGESIC DRUGS. PERCENTAGE OF MICE SHOWING ANALGESIA AFTER DAILY SUBCUTANEOUS INJECTIONS WITH DOSES CORRESPONDING TO ED80

Drug	% Analgesia		
	Before Treatment	Days from First Dose	
		10	22
Physiological saline ..	0/20	0/20	0/17
Morphine	15/20=75%	11/18=61%	3/12=25%
Pethidine	14/20=70%	11/20=55%	9/17=53%
(−)-Methadone ..	15/20=75%	16/20=80%	3/11=27%

TABLE V

ACUTE TOXICITY IN MICE: LD50 AND 19/20 CONFIDENCE LIMITS

Drug	Route of Administration			
	Subcutaneous		Intraperitoneal	
	LD50 mg./kg.	No. of Mice	LD50 mg./kg.	No. of Mice
Morphine ..	400 (367–436)	90	310 (269·5–356·5)	80
Pethidine ..	235 (216–256)	82	165 (155·6–174·9)	84
(±)-Methadone	46 (40–52·9)	65	28 (25·2–31)	100
(−)- ,,	41·3 (36·2–47·0)	66	32 (29·3–34·8)	80
(+)- ,,	80 (74–86·4)	100	74 (70·4–77·7)	110

The doses of all drugs are expressed in terms of the hydrochlorides.

TABLE VI

THERAPEUTIC INDEXES: LD50/ED50

Drug	Route of Administration	
	Subcutaneous	Intraperitoneal
Morphine	69·0	39·7
Pethidine	10·9	5·3
(±)-Methadone	18·4	6·3
(−)- ,, ...	22·9	11·0
(+)- ,, ...	2·0	2·0

TABLE VII

ANALGESIC ACTIVITY OF DRUGS TESTED BY VARIOUS METHODS IN DIFFERENT SPECIES

Drug	Noxious Stimulus Applied	Animals Used	Route of Administration	Equiactive Analgesic Doses (mg./kg.)	Relative Potency (Morphine = 1)	Reference
Morphine	Pressure	Mice	s.c.	1·4		Schaumann (1940)
Pethidine	,,	,,	,,	9·3	0·15	
Morphine	Heat	Rats	i.v.	2·2±0·13*		Davies, Raventos, and Walpole (1946)
			i.p.	10·0		
Pethidine	,,	,,	i.v.	4·6±0·59*	0·47	
			i.p.	30·0	0·33	
Pethidine	Pressure	Rats	i.p.	10·0		Scott and Chen (1946)
(±)-Methadone ..	,,	,,	,,	1·0		
(±)- ,, ...	Radiant heat	Dogs	,,	2·5		
Morphine	Radiant heat	Rats		2·0		Thorp, Walton, and Ofner (1947)
Pethidine	,, ,,	,,		15–20	0·15	
(−)-Methadone ..	,, ,,	,,		1·0	2·0	
Morphine	Radiant heat	Guinea-pigs	i.p.	12·1		Winder (1947)
Pethidine	,, ,,	,,	,,	52·8	0·23	
Morphine	Radiant heat	Rats	s.c.	3·01±0·21*		Cahen, Epstein, and Krementz (1948)
Pethidine	,, ,,	,,	,,	21±2·8*	0·14	
(±)-Methadone ..	,, ,,	,,	,,	2·5±0·28*	1·2	
(+)- ,, ..	,, ,,	,,	,,	82±0·80*	0·03	
(−)- ,, ..	,, ,,	,,	,,	1±0·32*	2·8	
Morphine	Radiant heat	Rats	s.c.	6		Randall and Lehmann (1948)
Methadone	,, ,,	,,	,,	6	1	
(+)-Methadone ..	Pressure	Rats	i.p.	30		Scott, Robbins, and Chen (1948)
(−)- ,, ..	,,	,,	,,	4		
(+)- ,, ..	Radiant heat	Dogs	s.c.	25		
(−)- ,, ..	,, ,,	,,	,,	1		
(±)-Methadone ..		Guinea-pigs	s.c.	12·5		Jenney and Pfeiffer (1948)
(−)- ,, ..		,,	,,	5·0		
(+)- ,, ..		,,	,,	20		
Morphine	Radiant heat	Rats	s.c.	3·5		Hougs-Olsen (1949)
Methadone	,, ,,	,,	,,	2·7	1·27	
Morphine	Radiant heat	Rats	s.c.	10·0		Lewis (1949)
(−)-Methadone ..	,, ,,	,,	,,	2·0	5	
Morphine	Radiant heat	Rats	s.c.	2·16±0·24*		Thorp (1949)
(±)-Methadone ..	,, ,,	,,	,,	1·58±0·19*	1·3	
(−)- ,, ..	,, ,,	,,	,,	1·02±0·10*	2·1	
(+)- ,, ..	,, ,,	,,	,,	0	0	
(±)-Methadone ..	Radiant heat	Rats	s.c.		1·3	Walton, Ofner and Thorp (1949)
(−)- ,, ..	,, ,,	,,	,,		2·2	
(+)- ,, ..	,, ,,	,,	,,		0	
Morphine	Radiant heat	Rats	i.p.	2·04		Bonnycastle and Ipsen (1950)
Pethidine	,, ,,	,,	,,	14·1	0·14	
Methadone	,, ,,	,,	,,	1·74	1·17	
Pethidine	Radiant heat	Rats	i.v.		0·05 or 0·09	Bonnycastle and Leonard (1950)
(±)-Methadone ..	,, ,,	,,	,,		1·14 or 0·83	
Morphine	Hot plate	Mice	s.c.	3·09±0·21*		Eddy, Touchberry, and Lieberman (1950)
(±)-Methadone ..	,, ,,	,,	,,	1·62±0·12*	1·90	
(−)- ,, ..	,, ,,	,,	,,	0·83±0·01*	3·72	
(+)- ,, ..	,, ,,	,,	,,	25·7±1·3*	0·12	
Morphine	Hot plate	Mice	s.c.	2·3		Herr and Pórszász (1950)
Pethidine	,, ,,	,,	,,	13 0	0·17	
Methadone	,, ,,	,,	,,	1·7	1·4	
Morphine	Hot plate	Mice	s.c.	5		Lespagnol, Mercier, Bertrand, and Mercier (1950)
Pethidine	,, ,,	,,	,,	20	0·25	
Morphine	Radiant heat	Rats	s.c.	4		Winter and Flataker (1950)
(±)-Methadone ..	,, ,,	,,	,,	2	2	
Pethidine	Radiant heat	Rats			0·24	Christensen and Tye (1951)
Methadone	,, ,,	,,			2·2	
Morphine	Radiant heat or pressure	Rats	s.c.	3·2		Green, Young, and Godfrey (1951)
Pethidine	,, ,,	,,	,,		0·20	
(±)-Methadone ..	,, ,,	,,	,,	1·5	2·1	

* ED50±S.E.

TABLE VII—*continued*

Drug	Noxious Stimulus Applied	Animals Used	Route of Administration	Equiactive Analgesic Doses (mg./kg.)	Relative Potency (Morphine = 1)	Reference
Morphine Methadone	Radiant heat " "	Rats "	s.c. "	2 2	 1	Petersen (1951)
Morphine Pethidine Methadone	Radiant heat " " " "	Rats " "	i.v. " "	2 10 1	 0·20 2	Bass and Vander Brook (1952)
Morphine Pethidine (±)-Methadone	Heat " "	Mice " "	s.c. " "	3·6 11·0 2·9	 0·33 1·25	Grewal (1952)
Morphine Pethidine Methadone	Heat " "	Rats " "	i.v. " "	2·0 (1·7–2·4) 2·0 (1·8–2·2) 0·31 (0·20–0·80)	 1 6·5	Jackson (1952)
Pethidine (±)-Methadone					0·15 1·5	Schaumann (1952)
Morphine Methadone	Radiant heat " "	Rats "	s.c. "	4·7 (4·2–5·2)† 1·9 (1·6–2·2)†	 2·5 (2·2–2·8)†	Tye and Christensen (1952)
Morphine (±)-Methadone Morphine (±)-Methadone	Hot plate " " " " " "	Mice " " "	s.c. " Oral "	2·1±0·1* 1·6±0·1* 3·9±0·5* 9·2±1·9*	 1·3 0·42	Eddy and Leimbach (1953)
Morphine Pethidine Methadone Morphine Pethidine Morphine Pethidine Methadone	Radiant heat " " " " " " " " Electric discharge " " " "	Mice " " Rats " Mice " "	s.c. " " " " " " "	1·7 8·6 1·4 6·8 16·3 4·5 4·5 2·5	 0·19 1·21 0·41 1 1·8	Haas, Hohagen, and Kollmannsperger (1953)
Morphine Methadone	Radiant heat " "	Mice "	s.c. "	2·30 (2·04–2·40)† 1·20 (1·03–1·40)†	 1·91	Kraushaar (1953)
Morphine (±)-Methadone Pethidine	Hot plate " " " "	Mice " "	s.c. " "	8·6 5·3 23·0	 1·6 0·37	Ohlsson (1953)
Morphine (±)-Methadone Pethidine	Hot plate " " " "	Rats " "	s.c. " "		 2·39 0·36	Pórszász, Tardos, Herr, and Nyiri (1953)
Morphine Pethidine Methadone	Heat " "	Mice " "	s.c. " "	5 15 1	 0·33 5	Serembe and Visentini (1953)
Morphine (±)-Methadone	Radiant heat " "	Rats "	s.c. "	4 1	 4	Smith and Lehman (1953)
Morphine Methadone	Hot plate " "	Mice "	s.c. "	5 2	 2·5	Jacob and Grassi-Gialdroni (1953)
(±)-Methadone (−)- " (+)- " (±)- " (−)- " (+)- "	Hot plate " " " " " " " " " "	Mice " " " " "	Oral " " s.c. " "	9·2 (7·3–11·6)† 8·0 (6·1–10·5)† 89·3 (56·6–141·0)† 1·6 (1·5–1·7)† 0·83 (0·82–0·84)† 25·7 (24·5–27·0)†		Leimbach and Eddy (1954)

* ED50±S.E. † ED50 and fiducial limits.

mice were injected subcutaneously with doses of drugs corresponding to ED80. The results are quoted in Table III. The analgesic action of morphine, pethidine, and (−)-methadone disappears in 3 hr.

In a third group of experiments we have determined the tolerance to the analgesic drugs. Mice were injected subcutaneously with ED80 for 22 days. The results are quoted in Table IV. It seems that tolerance is less pronounced for pethidine than for morphine or (−)-methadone.

The toxicities of these drugs are shown in Table V and their therapeutic indexes in Table VI.

DISCUSSION

Our data on the intensity of analgesic action of various compounds agree with those of others (reported in Table VII). (±)-Methadone is about twice and (−)-methadone is about three times as active as morphine; pethidine is about four times less active than morphine. In contrast to the findings of others, (+)-methadone was shown to have some analgesic action; injected intraperitoneally it is as active as pethidine. (−)-Methadone is about 1.5 times as active as (±)-methadone.

It appears from our data that the analgesic effect increases when drugs are injected subcutaneously.

It is known that the liver rapidly destroys analgesic drugs (Sung and Way, 1950; Richards, Boxer and Smith, 1950) and that the analgesic action is enhanced and prolonged by partial hepatectomy (Sung and Way, 1950; Bonnycastle and Delia, 1950). Since drugs injected intraperitoneally are absorbed by the portal system, they are presumably partly destroyed during passage through the liver, whereas drugs injected subcutaneously reach the central nervous system without passing through the liver. These considerations may explain the differences observed between intraperitoneal and subcutaneous administration.

Jackson (1952) suggests that the analgesic effect of drugs in the rat is not a manifestation of general toxicity. It would seem that this is also so in the mouse, since the toxicity of analgesic drugs is enhanced when they are injected intraperitoneally, while their analgesic effect is decreased.

Our data on the duration of action of, and tolerance to, the analgesic drugs agree with the results of others (Cahen, Epstein and Krementz, 1948; Lewis, 1949; Isbell, Wikler, Eddy, Wilson and Moran, 1947; Scott, Chen, Kohlstaedt, Robbins and Israel, 1947; Bass and Vander Brook, 1952). It can be concluded that the results obtained by Haffner's method are similar to those obtained by other methods of testing analgesic drugs.

Summary

1. A linear relation was found between log-dose and probit when analgesic activity was determined by Haffner's method in mice.

2. The analgesic effect of morphine, pethidine, (±)- and (−)-methadone increases when the drugs are injected subcutaneously instead of intraperitoneally. Acute toxicity decreases.

3. The results obtained for intensity, duration, and tolerance to morphine, pethidine, (±)-, (−)-, and (+)-methadone agree with those of other authors.

4. Haffner's method provides a simple means of testing analgesic drugs. In contrast to methods employing a thermal stimulus, Haffner's method is based on a reflex mechanism which involves the higher centres.

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