Effects of hexobarbitone, ether, morphine, and urethane upon the acute toxicity of propranolol and D-(—)-INPEA

SIR,—Recently, we reported that propranolol increased the acute toxicity of hexobarbitone (Murmann, Almirante & Saccani-Guelfi, 1966). The interaction of these drugs was attributed to the central nervous system depressant properties of propranolol since D(—)-2-isopropylamino-1-(*p*-nitrophenyl)ethanol(INPEA) another adrenergic β-receptor antagonist (Almirante & Murmann, 1966; Somani, 1966), did not interact with hexobarbitone, and D(—)-INPEA is known to be free from CNS depressant actions (Murmann, Almirante & Saccani-Guelfi, 1966). We have now extended these observations to include the interactions of propranolol and D-(—)-INPEA with ether, morphine and urethane.

To measure the median lethal doses of the various drugs, groups of twenty adult, male mice, NMRI strain, in a quiet room at 25 ± 0·2°, were given propranolol and D-(—)-INPEA subcutaneously and hexobarbitone (0·4%v/w) in 0·1N NaOH, ether (4% v/v), morphine (1·15% w/v) or urethane (7% w/v) in saline,

TABLE 1. EFFECT OF HEXOBARBITONE, ETHER, MORPHINE AND URETHANE ON THE TOXICITY OF TWO DIFFERENT β-ADRENERGIC BLOCKING AGENTS

Interval between β-blocker and test compound (min)	LD 50* mg/kg s.c.	Slope function	Time of death (range)	LD 50* mg/kg s.c.	Slope function	Time of death (range)
	Hexobarbitone: 40 mg/kg 0·6 LD 50 (intravenously) LD 50 = 65·6 (53·6–80·4) S = 1·39 (1·12–1·72)			Ether: 0·4 ml/kg 0·6 LD 50 (intravenously) LD 50 = 0·70 (0·63–0·78) S = 1·13 (0·87–1·46)		
Propranolol (control) ..	187·0 (164·5–212·6)	1·16 (1·03–1·31)	>1 <72 hr	240·0 (192·8–298·8)	1·42 (1·17–1·73)	>0·5 <2 hr
Simultan.	216·0 (186·1–250·8)	1·41 (1·06–1·89)	>1 <48 hr	279·0 (202·9–383·6)	1·86 (1·23–2·83)	>3 min <48 hr
15	35·2 (24·4–50·9)	3·33 (1·44–7·64)	>0·5 <7 min	11·4 (5·5–23·6)	5·20 (2·17–12·48)	>3 <7 min
30	41·8 (29·9–58·5)	1·92 (1·28–2·87)	>0·5 <6 min	25·2 (14·4–44·1)	2·97 (1·36–6·48)	>2 <7 min
60	38·5 (27·4–54·1)	1·75 (1·21–2·54)	>0·5 <5 min	47·0 (28·0–90·0)	2·28 (1·13–4·60)	>2 <12 min
120	36·0 (26·6–48·8)	1·64 (1·23–2·20)	>1 <6 min	52·0 (33·6–80·6)	1·68 (1·02–2·77)	>3 <6 min
180	50·8 (38·3–67·3)	1·38 (1·16–1·64)	>3 <11 min	78·0 (50·7–120·1)	2·37 (0·97–5·78)	>3 <7 min
300	73·5 (58·8–91·9)	1·29 (1·10–1·52)	>2 <11 min	>150		
480	122·5 (106·5–140·9)	1·18 (1·03–1·35)	>2 <16 min			
D(—)-INPEA (control) ..	322·0 (272·9–378·0)	1·39 (1·25–1·54)	>5 min <3 hr	290·0 (267·3–314·7)	1·24 (1·16–1·32)	>20 <60 min
Simultaneously	359·0 (320·5–402·1)	1·20 (1·10–1·31)	>5 min <16 hr	268·0 (245·9–292·1)	1·15 (1·08–1·23)	>2 <47 min
15	240·0 (210·7–273·4)	1·29 (1·06–1·58)	>3 <27 min	>200		
30	209·0 (166·5–262·3)	1·31 (1·05–1·63)	>1 <8 min	>200		
60	>300			>150		
120	>300					
	Morphine: 115 mg/kg 0·6 LD 50 (intravenously) LD 50 = 184·0 (160·7–210·7) S = 1·16 (1·02–1·33)			Urethane: 700 mg/kg 0·6 LD 50 (intravenously) LD 50 = 1130·0 (918·7–1389·9) S = 1·25 (1·03–1·52)		
Propranolol (control) ..	217·0 (192·9–244·1)	1·32 (1·21–1·43)	>0·5 <24 hr	217·0 (192·9–244·1)	1·32 (1·21–1·43)	>0·5 <24 hr
Simultaneously	107·0 (66·9–171·2)	2·85 (1·30–6·27)	>2 min <18 hr	116·0 (121·2–227·4)	1·44 (1·09–1·90)	>2 <280 min
15	31·5 (17·4–57·0)	3·83 (1·42–10·33)	>2 <8 min	0·228 (0·125–0·417)	3·27 (2·02–5·30)	>1 <6 min
30	41·0 (21·6–77·9)	3·66 (1·38–9·70)	>3 <10 min	1·000 (0·658–1·520)	2·28 (1·19–4·38)	>2 <7 min
60	55·8 (39·6–78·7)	1·98 (1·04–3·79)	>2 <8 min	0·740 (0·339–1·613)	6·64 (1·71–26·63	>1 <5 min
120	63·0 (38·4–103·3)	3·02 (1·26–7·24)	>2 <11 min	10·00 (6·64–15·05)	2·26 (1·51–3·39)	>1 <5 min
180	85·0 (49·4–168·3)	2·95 (1·33–6·54)	>1 <9 min	11·70 (5·04–27·14)	3·93 (1·40–11·00)	>2 <7 min
300	114·0 (81·4–159·6)	1·73 (1·19–2·53)	>2 <99 min	39·8 (26·53–59·7)	1·97 (1·47–2·65)	>1 <6 min
480				68·5 (51·7–90·8)	1·38 (1·11–1·62)	>3 <6 min
D(—)-INPEA (control)	322·0 (272·9–378·0)	1·39 (1·25–1·54)	>5 min <3 hr	322·0 (272·9–378·0)	1·39 (1·25–1·54)	>5 min <3 hr
Simultaneously	76·5 (51·0–114·8)	2·22 (1·11–4·44)	>2 min <18 hr	413·0 (384·2–444·0)	1·12 (1·02–1·24)	>25 <70 min
15	65·8 (47·2–91·8)	1·46 (1·15–1·86)	>1 min <24 hr	>300		
30	73·5 (51·7–104·7)	1·78 (1·11–2·85)	>2 <7 min	>300		
60	>150			>300		
120	>300					
180	>300					

* Results calculated from experimental data by the method of Litchfield & Wilcoxon (1949).

were given intravenously at the rate of 0·01 ml/sec. These agents were given in equivalent sub-lethal doses (0·6 LD50) either simultaneously or 15, 30, 60, 120, 180, 300, or 480 min after graded doses of propranolol or D(−)-INPEA; the LD50 estimates were calculated (Litchfield & Wilcoxon, 1949).

The three anaesthetics and morphine increased the toxicity of propranolol (Table 1). The enhanced toxicity of these drug combinations appeared to be associated with the interaction of CNS depressant actions. There was a significant increase in the sleeping times of the surviving animals when the anaesthetics and morphine were given after propranolol. By contrast, D(−)-INPEA is a CNS stimulant and greatly reduced the sleeping times they produced. While the anaesthetics did not enhance the toxicity of D-(−)-INPEA there was a clear interaction with morphine. This appeared to be due to the summation of their central excitatory actions. The central excitatory action of D-(−)-INPEA alone often led to convulsions and death when the higher dosed animals were handled for the administration of the test substances but when it was combined with the central excitatory action of morphine death by convulsions occurred at much lower doses of D(−)-INPEA. The interaction with morphine was thus quite different from the interactions of propranolol and the various test substances.

The electrocardiograms of mice were recorded to investigate the sudden deaths which occurred when the test substances were given after propranolol. The average heart rate of 25 normal, untreated, mice was 741 ± 12 beats per min. Doses of propranolol between 3–100 mg/kg subcutaneously reduced the heart rate of mice to between 350 and 450 beats per min. However, when the test substances were given 15 min after propranolol, an extreme bradycardia, ending in cardiac arrest, developed within a few minutes in each case.

Atropine was used to try to find the extent of vagal involvement in this bradycardia. Groups of 50 mice were treated with propranolol 2 or 3 mg/kg s.c. + urethane 700 mg/kg i.v. given 15 min after propranolol with or without atropine 2 mg/kg i.v. given at the same time as the propranolol. The atropine reduced but did not completely prevent the acute mortality produced by the urethane-propranolol combination (from 50 to 20% or from 86 to 40%). Since this dose of atropine would be expected to produce complete vagal blockade in the mouse, it would appear that either there is a substantial non-vagal component in the bradycardia or alternatively primary cardiac arrest is not the only cause of death. Propranolol is known to have both local anaesthetic actions and quinidine-like actions on the heart and so the effects of urethane on quinidine and xylocaine toxicity were investigated. As shown in Table 2, urethane

TABLE 2. EFFECT OF URETHANE ON THE TOXICITY OF QUINIDINE AND XYLOCAINE

Interval between administration of drugs (min)	Urethane: 700 mg/kg 0.6 LD50 (intravenously) LD50 = 1130.0 (918.7–1389.9) S = 1.25 (1.03–1.52)			
	LD 50* mg/kg s.c.	Slope function	Time of death (range)	
Quinidine (control) ..	465·0 (414·8–521·3)	1·20 (1·09–1·33)	>1	<16 hr
Simultaneously	389·0 (329·1–459·8)	1·21 (1·03–1·43)	>2	<24 hr
15	260·0 (208·0–325·0)	1·66 (1·33–2·07)	>2 min	<15 hr
30	235·0 (171·5–322·0)	1·58 (1·29–1·93)	>2 min	<9 min
Xylocaine (control) ..	264·0 (216·4–322·1)	1·25 (1·08–1·44)	>10	<90 min
Simultaneously	279·0 (216·3–359·9)	1·34 (1·02–1·76)	>5	<97 min
15	209·0 (185·8–235·1)	1·17 (1·04–1·32)	>2	<9 min
30	227·0 (174·6–295·1)	1·38 (1·02–1·86)	>2	<4 min

* Results calculated from experimental data by the method of Litchfield & Wilcoxon (1949).

produced a significant increase in quinidine toxicity but there was a barely significant interaction with xylocaine.

In conclusion, a marked synergism has been shown to occur between propranolol and various anaesthetics and morphine. This synergism was not seen with D(−)-INPEA. Unlike propranolol, D(−)-INPEA does not have depressant actions on the central nervous system nor quinidine-like actions on the heart—effects of propranolol which may be involved in its synergism with the anaesthetics and morphine.

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The effects of morphine, pethidine and nalorphine on the isolated frog skin preparation

SIR,—Although morphine has a long history of clinical use, the mechanism by which it exerts its important and complex effects upon the central nervous system is still obscure. As an alternative to direct studies on the neuraxis, experimentally simpler systems shown to be affected by morphine have been examined, e.g. guinea-pig ileum (Paton, 1957; Cox & Weinstock, 1966) and superior cervical ganglion (Kosterlitz & Wallis, 1966). The present work arose from the chance observation that morphine produced effects upon the frog skin preparation. The actions of some nitrogenous bases on the transport of sodium ions across this membrane have been studied by Kirschner (1953) and Skou (1961). In the following experiments, morphine, pethidine and nalorphine were applied to the isolated skin.

A circle of washed abdominal skin of *Rana temporaria* separated frog Ringer solution (pH 7·65) contained in two adjacent 15 ml cells at room temperature. The preparation was left for 2 hr to equilibrate and then the short-circuit current (SCC) which had to be applied to reduce the skin potential to zero was measured. The current was maintained continuously for the rest of the experimental period, adjustments and readings being made at 5 min intervals. Drugs were applied to either surface of the membrane and any changes in the SCC noted.

Fig. 1 shows typical results following application to the inside of the skin. The three drugs produced significant falls in SCC, approximately equipotent doses being morphine sulphate 10 mg, pethidine hydrochloride 1 mg and nalorphine hydrobromide 5 mg (corresponding to the following final concentrations in the bathing solution in terms of the free bases: morphine 1·75 mM, pethidine 0·23 mM and nalorphine 0·85 mM). Although different preparations varied in sensitivity, the initial value of the SCC did not appear to be critical provided that it was greater than 80 μA/4 cm^2. When the same drugs were applied to the outer surface of the frog skin, larger doses (4–10 times) were required to produce significant falls in the SCC. It will be seen from Fig. 1