Increased toxicity of morphine-like analgesics in aggregated mice

The early observations of an enhanced excitation (Gunn & Gurd, 1940) and increased lethality (Chance, 1946) after amphetamine in aggregated mice stimulated much subsequent investigation of the aggregation phenomenon. Thus, Greenblatt & Osterberg (1961) found that for aggregated mice the LD50 for some stimulants such as caffeine, picrotoxin or amiphenazole, and several phenethylamine derivatives, was significantly lower than that for isolated mice, but this was not so after phenelzine, mescaline, ephedrine, leptazol and bemegride. The stimulant activity of morphine in mice at the spinal cord level is well known from the Straub-tail phenomenon, and at the brain level by an enhancement of locomotor activity. The latter effect of morphine is antagonized by pretreatment with α-methyltyrosine (AMT), a finding that points to the morphine-induced activity being the result of a catecholamine-altering mechanism (Menon, Dandiya & Bapna, 1967). While Spoerlein (1968) has reported finding a difference in the acute LD50 of morphine for mice between aggregated and isolated conditions, Vedernikov (1970) found no difference in such a test. These considerations have led us to examine the influence of aggregation on the lethal dosage of several morphine-like analgesics and one morphine-like antagonist-analgesic, and to test combinations of AMT and the analgesics under similar conditions.

Swiss albino random-bred male mice of 26 to 32 g were housed for 7–10 days before experiments in plastic boxes (45 × 24 × 12·5 cm), 20–25 mice to a box. After being injected intraperitoneally they were placed in stainless steel cages (18 × 10 × 12·5 cm), either 1 or 5 to a cage. Three sides and the top were solid while the floor and front of the cage were of wire mesh. Preliminary results were the basis for selecting the 5 dosages used in each LD50 determination. Fifteen mice were treated at each dose level. Injections were begun at mid-morning and the mice were checked for deaths every half hour for the first 4 h and also at 12 and 24 h. Aggregated mice that died were removed and replaced by marked, untreated mice to maintain aggregation. Four h mortality figures were used to calculate LD50 values (Litchfield & Wilcoxon, 1949).

All test drugs significantly enhanced the acute lethality among aggregated animals, i.e. all had isolated: aggregated potency ratios significantly greater than one (Table 1). Although the ratios were statistically significant, several were so only by a slight

Table 1. *Effect of aggregation on lethality of mice treated with (+)-amphetamine or with analgesics.* Mice were aggregated in groups of 5 per cage. Fifteen mice were treated at 5 dose levels for each drug in both aggregated and isolated conditions. Figures in parentheses are 95% confidence limits.

Drug	Isolated LD50	Aggregated LD50	Potency ratio
(+)-Amphetamine sulphate	27·5 (25·1–30·1)	9·15 (8·36–10·0)	3·01 (2·68–3·37)
Levorphanol tartrate	146 (139–153)	106 (98·2–114)	1·38 (1·26–1·51)
Meperidine hydrochloride..	139 (125–155)	110 (98–124)	1·26 (1·07–1·49)
Methadone hydrochloride..	48·0 (45·7–50·4)	27·7 (25·4–30·2)	1·73 (1·58–1·90)
Morphine sulphate	470 (446–496)	413 (384–445)	1·14 (1·09–1·19)
Pentazocine lactate..	103 (97·9–109)	93·5 (90·0–97·0)	1·11 (1·06–1·15)

margin, pentazocine, morphine and meperidine having potency ratios of less than 1·30. On another occasion, however, we have observed a potency ratio for morphine as high as 1·62 (1·35–1·94). On the other hand, the values found for levorphanol and methadone were 1·38 and 1·73, respectively. All of these potency ratios are significantly less than that for (+)-amphetamine, 3·01. In no comparison was there a significant difference in the slopes of the regression lines for aggregated and isolated lethality.

Whether the enhancement of toxicity by aggregation is dependent upon a release of brain catecholamines, was challenged by testing the ability of (±)-α-methyl-*p*-tyrosine (AMT) to counteract the aggregation effect for all five analgesics and for (+)-amphetamine. Methods were as described above except that only a single dose level of the test drugs was used, the incidence of mortality of saline plus test drug to AMT + test drug being compared by the χ^2 test (Siegel, 1956). Two doses of AMT, 50 mg/kg each, were administered as a suspension in saline at 12 and 4 h before injection of the test drug.

No reduction of lethal toxicity of the analgesics was produced by AMT, although the dosage schedule was effective in protecting mice treated with (+)-amphetamine. Protection against amphetamine-induced lethality was highly significant even with only a single 50 mg/kg dose of AMT at the 4 h pretreatment time (10/25 deaths for AMT–amphetamine and 21/24 deaths after saline–amphetamine). The 2-dose AMT schedule was found to have less effect on lethality of (+)-amphetamine in isolated mice (3/25 *vs* 6/25 deaths 4 h after 35 mg/kg of (+)-amphetamine, and 4/25 *vs* 10/25 at 10 h). These results are in accord with the report of Spoerlein (1968) in which she stated that several catecholamine-modifying agents failed to antagonize the lethality of morphine in grouped mice. Agents she tried were reserpine, chlorpromazine, α-methyl-*m*-tyrosine, α-methyldopa, tolazoline, phenoxybenzamine, propranolol, tyramine, guanethidine, imipramine and phenelzine. As expected, the results of the AMT-amphetamine combination are in accord with results reported previously on this interaction (Mennear & Rudzik, 1966; Menon & Dandiya, 1967).

To test for the possible involvement of 5-hydroxytryptamine in the aggregation lethality phenomenon of the analgesics, *p*-chlorophenylalanine (PCPA) pretreatment was given before test doses of morphine and meperidine. The dosage schedule of 100 mg/kg of PCPA repeated 3 times at 72, 48 and 24 h before the analgesics gave no sign of altering the aggregated lethality of analgesics. This result is of interest in view of the recent report of Jounela (1970) that PCPA pretreatment was able to antagonize the combined lethality of phenelzine and meperidine in aggregated mice. However, he did not test whether such pretreatment could modify toxicity of meperidine alone.

Whereas Jounela's results indicate that a 5-HT system is involved in the synergistic interaction of MAO inhibitors and narcotic analgesics, it does not appear that such a system is relevant to the aggregated lethality of mice with such analgesics alone. Our AMT–morphine evidence also appears to exclude any role of a catecholamine-related mechanism in this aggregated lethality response, a circumstance by which the response can be clearly differentiated from the aggregation lethality of (+)-amphetamine.

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A possible synaptic mechanism underlying the similar behavioural effects of adrenaline-like and acetylcholine-like drugs

There are many situations in which adrenaline-like and acetylcholine-like compounds produce similar effects. Amphetamine, a drug that increases activity at adrenoceptive synapses by preventing the reuptake of released noradrenaline (Rutledge, 1970), has behavioural effects very similar to those produced by the two closely related muscarinic blockers hyoscine and atropine. Both amphetamine and atropine disrupt timing behaviour on Fixed Interval (Ray & Bivens, 1968) and on differential reinforcement of low rates of response (Carlton, 1963; Bivens & Ray, 1968) operant conditioning schedules; both increase response rates on a Sidman avoidance schedule (Carlton, 1963; Ray & Bivens, 1968); and both increase responding during periods of non-reward in a discrete trial bar press task (Heise, Laughlin & Keller, 1970). A behavioural tolerance is quickly formed to chronic doses of both amphetamine (Schuster & Zimmerman, 1961) and hyoscine (Bignami & Gatti, 1968) in situations where the initial drug effect is a reduction in reinforcement. This suggests that the same behavioural effects produced by adrenergic stimulation and cholinergic blockade could arise from a common neural mechanism rather than necessarily from two complimentary neural systems.

The existence of a common mechanism is also suggested by the demonstration that subthreshold doses of atropine and amphetamine, when given simultaneously, act additively to give the same effect as do larger doses of either drug given alone (Carlton, 1963; Bivens & Ray, 1968; Ray & Bivens, 1968). Although these facts might be reconciled by postulating reciprocally acting adrenergic and cholinergic systems with adrenergic stimulation having the same behavioural effect as cholinergic blockade, the demonstration that adrenergic activation also produces the same effect as cholinergic activation does not fit the reciprocally acting systems hypothesis. Intracranial self-stimulation of the lateral hypothalamus is depressed by adrenaline (Mogenson, Russek & Stevenson, 1969), by noradrenaline (Olds, Yuwiler & others, 1964), by amphetamine (Umemoto & Kido, 1967) as well as by the centrally-active cholinergic agonist physostigmine (Domino & Olds, 1968; Stark, Totty & others, 1968; Olds & Domino, 1969a, 1969b). The lateral hypothalamus in turn makes adrenergic inhibitory synapses in the amygdala (Stein & Wise, 1969) and cholinergic inhibitory