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The effect of reserpine and α-methyldopa on the analgesic action of morphine in the mouse

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In the tail clip test, reserpine inhibited the analgesic action of morphine, and this action of reserpine was prevented by pretreatment with α-methyldopa. In the hot plate test reserpine potentiated the action of morphine, and α-methyldopa pretreatment had no inhibitory action on reserpine. α-Methyldopa alone, and in combination with reserpine, showed an analgesic action in the hot plate test.

THE effect of reserpine on morphine analgesia has been widely studied; some reports describe an inhibitory action of reserpine, others a potentiating effect. Antagonism in mice was demonstrated by Schneider (1954), Schaumann (1958), Sigg, Caprio & Schneider (1958), Tsou Kong & Tu Zeng-Hong (1963), Medakovic & Banic (1964) and Takagi, Takashima & Kimura (1964). On the other hand, Tripod & Gross (1957), Garcia Leme & Rocha e Silva (1961) and Dandiya & Menon (1963) reported that reserpine enhanced morphine analgesia.

In our hands, the nature of the effect of reserpine on morphine in mice was dependent on the method used to demonstrate analgesia. Whereas reserpine antagonized the action of morphine in the tail clip test it potentiated morphine when thermal stimulation of the paw was used. These results are now reported.

Experimental

Male Schneider mice, 18–22 g, were housed in groups of 5. Tail clip and hot plate tests were made on the same animals, with additional mice occasionally being used in either test.

TAIL CLIP TEST (Bianchi & Franceschini, 1954)

An artery clip covered in plastic tubing was applied to the base of the tail for a period not exceeding 10 sec. Mice were tested at intervals of 10 min after the injection of morphine, for a period of 50 min. Those reacting by biting the clip or by rapid backward movements, were regarded as showing a positive response. A negative response was shown by a characteristic state of immobility when the clip was in position.

HOT PLATE TEST (based on the method of Eddy & Leimbach, 1953)

This was done in a copper histological embedding bath in which the water temperature was held at 55°. Mice were placed on the hot plate at 30 min intervals after the injection of morphine during a period of 2 hr. Those that reacted by licking the front paws or by jumping out of the bath during a 15 sec period, were classed as positive.

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DRUGS

L-α-Methyldopa. 100 or 400 mg/kg, intraperitoneally, was administered 2 hr before reserpine and 4 hr before morphine. *Reserpine.* Serpasil, 2·5 mg/kg subcutaneously, was injected at either 15 min, 2, 24 or 48 hr before morphine. Alternatively a dose of 1·0 mg/kg, subcutaneously, was given 2 hr before morphine. *Morphine hydrochloride* was administered intraperitoneally at doses of 20, 10 or 5 mg/kg. All drugs were made up in saline with the exception of reserpine which was diluted with water. The dose volume was 0·2 ml/20 g. Controls received saline at the appropriate times. P values were calculated by the χ^2 test.

Results

Effect of reserpine, α-methyldopa alone or α-methyldopa followed by reserpine (Table 1). In the tail clip test, neither reserpine nor α-methyldopa caused analgesia and the action of α-methyldopa was not modified by a subsequent injection of reserpine.

TABLE 1. EFFECT OF RESERPINE, α-METHYLDOPA ALONE OR FOLLOWED BY RESERPINE

Pretreatment and time before analgesic test	Tail clip % analgesia						Hot plate % analgesia				
	No. mice	Time (min) 10	20	30	40	50	No. mice	Time (min) 30	60	90	120
Saline s.c.	70	0	0	0	0	0	70	3	16	13	14
Reserpine 2·5 mg/kg, s.c., 15 min	20	5	0	0	0	0	20	0	5	5	5
" " 2 hr	35	0	0	0	0	0	35	0	9	9	14
" " 24 hr	20	0	0	0	0	0	20	35[1]	20	30	30
" " 48 hr	25	0	0	0	0	0	25	4	4	20	36[3]
Saline i.p. 4 hr. Saline s.c. 2 hr	20	0	0	0	0	0	20	0	10	5	15
α-Methyldopa 100 mg/kg, i.p. 4 hr. " " "	20	0	0	0	0	0	20	5	10	0	10
α-Methyldopa 400 mg/kg, i.p. 4 hr " " "	20	10	10	5	0	5	20	0	20	60[1]	55[1]
α-Methyldopa 100 mg/kg, i.p. 4 hr. Reserpine 2·5 mg/kg, s.c. 2 hr	20	5	0	0	0	0	20	20[2]	35	65[1]	90[1]
α-Methyldopa 400 mg/kg, i.p. 4 hr. Reserpine 2·5 mg/kg, s.c. 2 hr	20	5	5	15	5	5	20	20[2]	55[1]	65[1]	80[1]

P values refer to comparison with saline control. [1] <0·001; [2] <0·01; [3] <0·05.

In the hot plate test, reserpine showed a slight analgesic action when given 24 or 48 hr before the test but none at shorter pretreatment times. α-Methyldopa, however, although inactive at 100 mg/kg, had a strong analgesic action at 400 mg/kg. This activity was evident only at the 90 and 120 min test periods, that is at 5·5 and 6 hr after injection. When α-methyldopa at either dose level was followed by reserpine, a marked analgesic effect was obtained.

Effect of reserpine or α-methyldopa on morphine (Table 2). In the tail clip test, reserpine either abolished or much reduced the action of morphine at all pretreatment times, whereas α-methyldopa had no significant effect.

In the hot plate test reserpine greatly enhanced the effect of the 20 or 10 mg/kg dose of morphine. Potentiation was evident at all pretreatment times from 15 min to 48 hr. α-Methyldopa did not affect the action of morphine.

TABLE 2. EFFECT OF RESERPINE OR α-METHYLDOPA ON MORPHINE

Pretreatment and time before morphine	Morphine mg/kg, i.p.	Tail clip: No. mice	Tail clip: % showing analgesia, Time (min) 10	20	30	40	50	Hot plate: No. mice	Hot plate: % showing analgesia, Time (min) 30	60	90	120
Saline s.c., 15 min	20	20	40	55	50	25	25	25	36	24	12	4
	10	20	20	30	30	20	5	20	15	5	0	7
	5	20	5	10	5	5	0	20	0	0	0	5
Reserpine 2·5 mg/kg, s.c., 15 min	20	20	15[4]	10[2]	5[2]	5	0[3]	25	36	68[2]	48[2]	52[1]
	10	20	0[4]	0[2]	0[2]	0[4]	0	20	25	15	30[2]	27
	5	20	0	0	0	0	0	20	5	15	15	5
Saline s.c., 2 hr	20	110	43	52	52	52	45	105	46	38	26	22
	10	55	14	22	24	20	7	55	13	24	13	16
	5	30	7	3	3	0	0	20	10	15	15	25
Reserpine 2·5 mg/kg, s.c., 2 hr	20	75	4[1]	7[1]	4[1]	1[1]	0[1]	80	65[3]	72[1]	75[1]	67[1]
	10	35	0[3]	0[2]	0[2]	0[2]	0	45	64[1]	56[2]	53[1]	60[1]
	5	20	0	0	0	0	0	20	15	40	40	35
Reserpine 1·0 mg/kg, s.c., 2 hr	20	15	7[3]	7[1]	0[1]	0[1]	0[1]	15	53	40	47	13
	10	20	0	0[4]	0[2]	0[4]	0	20	30	35	50[1]	67[1]
Saline s.c., 24 hr	20	10	50	40	50	40	30	10	50	20	30	40
Reserpine 2·5 mg/kg, s.c., 24 hr	20	20	0[1]	0[2]	0[1]	0[2]	0[2]	20	75	65[3]	80[2]	75
Saline s.c., 48 hr	20	10	70	40	50	50	50	10	20	30	10	10
Reserpine 2·5 mg/kg, s.c., 48 hr	20	20	10[1]	5[3]	5[2]	15[4]	5[2]	20	60[4]	80[2]	75[1]	70[2]
Saline i.p., 4 hr	20	20	45	65	65	50	60	20	30	30	45	25
	10	30	27	40	30	27	27	50	14	36	24	30
α-Methyldopa 100 mg/kg, i.p. 4 hr	20	20	70	55	65	70	65	20	35	30	30	15
	10	20	55[4]	45	40	45	30	20	35	35	30	35
α-Methyldopa 400 mg/kg, i.p. 4 hr	20	20	50	55	50	60	55	20	40	35	50	35

P values refer to a comparison with the saline pretreated morphine control. [1] <0·001; [2] <0·01; [3] <0·02; [4] <0·05.

Effect of α-methyldopa followed by reserpine on morphine (Table 3). In the tail clip test, α-methyldopa prevented the inhibitory effect of reserpine on morphine, while in the hot plate test, morphine preceded by α-methyldopa and reserpine caused pronounced analgesia. This can be attributed to an additive effect between morphine on the one hand and α-methyldopa and reserpine on the other.

TABLE 3. EFFECT OF α-METHYLDOPA, FOLLOWED BY RESERPINE, ON MORPHINE

Pretreatment: −4 hr, i.p.	Pretreatment: −2 hr, s.c.	Morphine mg/kg, i.p.	Tail clip test: No. mice	Tail clip test: % showing analgesia, Time (min) 10	20	30	40	50	Hot plate test: No. mice	Hot plate test: % showing analgesia, Time (min) 30	60	90	120
Saline	Saline	20	20	45[3]	65[1]	65[1]	50[2]	60[2]	20	30[3]	30[4]	45	25[4]
Saline	Saline	10	30	27[2]	40[1]	30[2]	27[2]	27[2]	50	14[1]	36	24	30
Saline	Reserpine	20	10	0	0	0	0	0	20	70	65	65	60
Saline	Reserpine	10	30	0	0	0	0	0	50	52	48	42	28
α-Methyldopa 100 mg/kg	Reserpine	20	20	65[1]	40[3]	60[2]	80[1]	80[1]	30	70	87	93[3]	87[4]
α-Methyldopa 400 mg/kg	Reserpine	20	20	45[3]	45[3]	45[3]	50[2]	45[3]	20	65	65	90	90[4]
α-Methyldopa 100 mg/kg	Reserpine	10	20	20[3]	20[3]	20[3]	20[3]	15[4]	20	60	65	75[3]	85[1]

Reserpine 2·5 mg/kg s/c.
P values refer to a comparison with saline, reserpine, morphine pretreated mice. [1] <0·001; [2] <0·001; [3] <0·02; [4] <0·05.

Discussion

In the same mice, reserpine showed a marked antagonism to morphine in the tail clip test and potentiation in the hot plate test. Antagonism in the former test confirms the results of Schaumann (1958), Tsou Kong & Tu Zeng-Hong (1963) and Takagi & others (1964), and potentiation in the hot plate test is in agreement with the results of Garcia Leme & Rocha e Silva (1961—hot plate) and Dandiya & Menon (1963—hot wire) but not with those of Medakovic & Banic (1964—hot plate), Sigg & others (1958—hot wire) and Schneider (1954—heat on tail). It is interesting to note that whereas there is unanimous agreement between different workers on the interaction of reserpine and morphine in the tail clip test, results differ when methods based on heat are used.

It would appear from these results that different mechanisms are involved in the nociceptive response to mechanical compression of the tail and thermal stimulation of the paw, and this conclusion is supported by the action of α-methyldopa in antagonizing reserpine in the former test and being synergistic with it in the latter. Inhibition of morphine by reserpine is probably a central effect since tetrabenazine, which also reduces the action of morphine in the tail clip test (Takagi & others, 1964) has little peripheral action (Pletscher, 1957; Quinn, Shore & Brodie, 1959). In addition reserpine is known to inhibit another central effect of morphine, namely psychomotor stimulation (Tripod, Bein & Meier, 1954; Tripod & Gross, 1957).

Potentiation could also result from a central effect if different receptors are involved and certainly the complex coordinated behaviour that serves as an endpoint in both tests is susceptible to modification at several points. However, a peripheral action of the drugs, as an explanation of the hot plate results, cannot be ruled out. A heat stimulus may be more readily antagonized at the periphery than mechanical compression since, with the former, a release of chemical mediators has been described.

There is evidence that bradykinin is released in response to heat injury of the rat paw (Rocha e Silva & Antonio, 1960) and that kinins may be released in response to a 20 sec burn of the guinea-pig foot at 55° (Davies & Lowe, 1966). The ability of this substance to cause pain is well known (Armstrong, Jepson & others, 1957; Elliott, Horton & Lewis, 1960), and it is not inconceivable that the nociceptive response to heat is initiated by release of a bradykinin-like substance. Rocha e Silva (1962) speculated that liberation of catecholamines from peripheral sites preceded the activation of bradykininogen and formation of bradykinin after local heating, and showed that pretreatment with reserpine inhibited the resulting inflammatory response. Winder's (1959) suggestion that an analgesic effect could result from interference with preinflammatory pain-producing substances at the site of injury may explain how the interaction of reserpine and morphine and of reserpine and α-methyldopa produces results in the hot plate test that are opposed to those obtained by the tail clip method.

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