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Abstract

The analgesic effects of morphine and some related compounds, such as meperidine, observed by the conventional method, are supplemented by the release of epinephrine from the adrenal medulla. It is assumed that this action of epinephrine is not due to an additive synergy in the analgesic effect but to the face that the action of epinephrine on a definite higher center or centers effects synergistically in the reflex depressant action of these analgesic agants. This assumption is based on the following evidences. Prolongation of reaction time in mice by morphine and meperidine (but not by ohton), determined by the hot-plate method, was significantly reduced by adrenalectomy and this reduction was normalized by the concurrent use of epinephrine, in a small dose which in itself cannot prolong the reaction time. No such action was found in cortisone and DOCA. The effects of morphine and meperidine in prolonging the reaction time were reduced by priscol and dibenamine, as well as by tetraethylammonium salt. A large dose of pyrazolone derivatives causes, not the prolongation of reaction time but a jumping reflex response in the early stages, indicating central excitation, in part of the mice. The ratio of mice exhibiting such an early reflex increases with adrenalectomy or the administration of dibenamine, and is markedly decreased by epinephrine, insufficient to show any analgesic response by itself, and by cortisone. This action of cortisone indicates some difference in the natures of central excitation by pyrazolones and by morphine. Judging from the work of SCHAYER18, the distribution in the brain of epinephrine injected in the dose to normalize the reduced effect of morphine in the adrenalectomized mice, may also be anticipated by the epinephrine which might be released from the adrenal medulla by morphine in an amount much smaller than the “near·lethal doses9 ”.

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THE SIGNIFICANCE OF THE ADRENAL MEDULLARY EPINEPHRINE IN THE ANALGESIC EFFECTS OF MORPHINE AND A FEW OTHER DRUGS IN MICE

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In recent years, some workers[1, 2, 3, 4] have suggested that morphine analgesia the whole or in part is mediated by the release of epinephrine from the adrenal medulla. This hypothesis is based on the facts that morphine causes release of epinephrine from the adrenal medulla[5, 6], that epinephrine itself shows an analgesic effect[3, 7, 8], and that the elevation of the pain-reaction threshold by morphine-type analgesics in dogs and rats falls after adrenalectomy[2, 3, 4].

However, MILLER *et al.*[9] recently reported that adrenalectomy failed to decrease the rise of the pain-reaction threshold by morphine but rather increased it. There are also observations unfavorable for the epinephrine hypothesis that some sympatholytic agents do not reverse the analgesic effect of morphine and methadone[3, 10] and that tetraethylammonium salt, which inhibits epinephrine release from the adrenal medulla[11] does not reverse the analgesic effect of morphine but rather strengthenes it[9]. Another reason for the opposition of MILLER *et al.*[9] to this hypothesis is that in their findings the amount of epinephrine actually liberated from the adrenal medulla in response to morphine has been in itself not enough to cause analgesia. However, no one has yet given any sufficient proof in support of the premise to the argument, that the action of epinephrine involved in the analgesic mechanism of morphine is of a simple additive nature with regard to the analgesic effect.

It has been found recently that, with the use of the simple hot-plate apparatus devised by SANUKI and OHNO[12] with selected mice, a comparatively delicate difference in analgesic effects can be well reproduced even with a small number of animals. As the experiments by the aforementioned workers have been carried out, using animals other than mice, the reexamination of this problem with foregoing method deemed necessary. Therefore, in order to obtain some definite answer to the present problem

some observations have been made to see whether or not adrenalectomy has any influence on the analgesic effects of morphine, and further tried to see if there is any influence on the analgesic effects of other drugs such as meperidine, ohton (3-dimethylamino-1, 1-di(2'-thienyl) but-1-ene hydrochloride), and a few derivatives of pyrazolone, besides morphine.

METHODS

Male mice, weighing 15±3 g. were used in a series of present experiments performed during September and October. The simplified hot plate apparatus of SANUKI and OHNO[12] already described, was used for algesimetry. The temperature of the metal plate was regulated to 56±0.5°C. One mouse each was placed gently on the metal plate and the time (in seconds) required until the mouse reacted to heat stimulation by the signs of discomfort such as bending or raising, shaking of the hind limbs, licking of the hind paws, and dancing about or attempts to jump out of the restraining glass cylinder, was measured with a stop watch. As a rule, the average value for 12 mice comprising each group was taken as the mean reaction time. Reaction time was tentatively measured twice at intervals of 1 hour and those mice showing the reaction time of 7-12 seconds in each test were selected for the experiments. Such mice comprised approximately 90% of the commercial animals tested at random. This reaction time remained almost unchanged even after adrenalectomy.

For the determination of analgesic effect of drugs, the reaction time was measured twice before the injection at intervals of 15 minutes, and 15, 30, 45, 60, 90 and 120 minutes after the injection. The difference between the maximum reaction time after the injection and the mean value of the two reaction times before the injection, i. e. the average of maximum prolongation of the reaction time was taken of each individual, and this value was used for the comparison of the effect, because the reaction time before the injection did not show any significant difference for all the mice used for the present experiments (cf. Table 1).

Bilateral adrenalectomy was performed by standard procedure by the lumbar approach under aseptic precautions. The groups of mice submitted to laparotomy and exposure of adrenal glands but not their removal were employed as the sham-operated controls. The adrenalectomized animals were given 1 % of sodium chloride in their drinking water and some groups received in addition two subcutaneous injections of cortisone acetate in 0.1 mg./10 g. dose during 24 hours before the experiment and one of the other groups cortisone acetate 0.1 mg./10 g. together with desoxycorticosterone acetate (DOCA) 0.02 mg./10 g. once 24 hours before. A

number of animals not receiving cortisone died within one week after the operation but those receiving it survived for a longer period. Experiments with adrenalectomized mice were carried out for 24—48 hours after the operation.

All the drugs were dissolved in 0.9% saline solution except DOCA given in oil and 0.1—0.2 c.c. of such a solution, containing the required dosage, was administered subcutaneously on the back. Concurrent administration of two different drugs was made on different sites.

RESULTS

The results obtained are summarized in Table 1.

Morphine. Throughout the experiment, the effect of subcutaneous injection of 0.1 mg./10 g. of morphine hydrochloride was observed. In normal intact mice, the maximum prologation of mean reaction time reached 16.0 sec. 30 minutes after the injection and gradually returned to the preinjection reaction threshold over two hours. This increase of reaction time resembled that in the sham-operated mice; no significant difference could be observed in the effect of these two groups of mice. In the adrenalectomized mice, with or without cortisone injection, the increase of mean reaction time was around 9.5 sec., indicating a marked reduction from the former two groups. These two adrenalectomized groups also differed from the sham-operated or intact mice in that the maximum increase was reached 45 minutes after the injection, 15 minutes later than the latter groups. DOCA could not alter the response of the adrenalectomized mice treated with cortisone. This also indicates that the difference of morphine analgesia by the presence or absence of the adrenal glands does not depend on cortisone and DOCA.

One group of sham-operated mice was given 1 μg./10 g. of epinephrine and another group was given 2 μg./10 g. of *dl*-norepinephrine subcutaneously with morphine. The effect of morphine showed only a slight, statistically insignificant, additional increase by the concurrent use of either drug. On the other hand, the effect of the concurrent use of these drugs was marked against the reduced effect of morphine in the adrenalectomized groups. With 1 μg./10 g. of epinephrine, the reduced effect of morphine was completely recovered and brought an increase in reaction time, equalling the effect of morphine injected in the sham-operated mice. This effect of epinephrine was observed fairly markedly even at 0.1 μg./10 g. and the maximum effect occurred 30 minutes after the injection. This action was much weaker in norepinephrine. The injection of 1 μg./10 g. of epinephrine alone failed to cause practically any change in the mean reaction

Table 1. Effects of epinephrine and some other drugs on the analgesic effect of morphine and a few other analgesics in normal and adrenalectomized mice

Analgesics (Dose per 10 g.)	Combined treatment (Dose per 10 g.)	Kind of mice	Preinjection M. R. T. (sec.)	Maximum prolongation of M. R. T. and 19/20 confidence limits (sec.)	No. of mice
Morphine 0.1 mg.	—	N	9.0 ± 0.22	16.0 (14.2 — 17.8)	12
	—	S	9.1 ± 0.19	15.1 (14.0 — 16.2)	12
	—	A	9.2 ± 0.24	9.7 (8.0 — 11.4)	12
	Cortisone 0.1 mg. ×2	A	9.3 ± 0.30	9.4 (7.9 — 10.9)	12
	Cortisone 0.1 mg. + DOCA 0.02 mg.	A	9.3 ± 0.24	9.8 (8.6 — 11.0)	12
	Epinephrine 1 μg.	S	8.9 ± 0.22	16.7 (15.9 — 17.5)	12
	Epinephrine 0.1 μg.	A	9.3 ± 0.24	14.1 (13.1 — 15.1)	12
	Epinephrine 1 μg.	A	9.1 ± 0.21	15.7 (14.9 — 16.5)	12
	Norepinephrine 2 μg.	S	8.8 ± 0.20	16.5 (15.5 — 17.5)	12
	Norepinephrine 1 μg.	A	8.9 ± 0.21	11.8 (10.9 — 12.7)	12
	Norepinephrine 2 μg.	A	8.6 ± 0.27	13.2 (12.1 — 14.3)	12
	Priscol 0.05 mg.	N	9.3 ± 0.29	10.9 (9.7 — 12.1)	12
	Priscol 0.05 mg.	A	8.7 ± 0.22	8.3 (7.5 — 9.1)	12
	Dibenamine 0.1 mg.	N	9.0 ± 0.23	10.4 (9.1 — 11.7)	12
	Dibenamine 0.1 mg.	A	9.0 ± 0.24	8.2 (7.3 — 9.1)	12
	T E A 0.5 mg.	N	8.6 ± 0.25	14.0 (12.9 — 15.1)	12
	Epinephrine 1 μg.	N	9.2 ± 0.22	0.2 (−0.8 — 1.2)	12
	Epinephrine 1 μg.	A	8.9 ± 0.20	0.4 (−0.1 — 0.9)	12
Meperidine 0.2 mg.	—	N	9.3 ± 0.24	10.2 (9.1 — 11.3)	12
	—	S	9.0 ± 0.20	10.0 (9.2 — 10.8)	12
	—	A	9.5 ± 0.22	5.9 (4.8 — 7.0)	12
	Epinephrine 1 μg.	A	9.3 ± 0.22	9.4 (8.2 — 10.6)	12
	Dibenamine 0.1 mg.	N	9.0 ± 0.26	7.6 (6.6 — 8.6)	12
Ohton 0.08 mg.	—	N	9.3 ± 0.21	16.7 (15.3 — 18.1)	12
	—	A	9.0 ± 0.24	17.3 (16.2 — 18.4)	12
	Dibenamine 0.1 mg.	N	8.6 ± 0.24	17.0 (15.9 — 18.1)	12
	T E A 0.5 mg.	N	8.7 ± 0.21	16.8 (15.8 — 17.8)	12
Aminopyrine 1 mg.	—	N	8.9 ± 0.23	11.7 (10.2 — 13.2)	12
	—	A	9.3 ± 0.16	11.5(−1.2 — 24.2)	2
				−6.1(−7.4 —−4.8)	10*
	Cortisone 0.1 mg. ×2	A	8.9 ± 0.29	10.7 (8.4 — 13.0)	12
	Epinephrine 1 μg.	A	9.2 ± 0.23	11.2 (9.9 — 12.5)	12
	Dibenamine 0.1 mg.	N	9.0 ± 0.22	11.2 (10.0 — 12.4)	8
				−3.1(−5.8 —−0.4)	4*
Aminopyrine 2.5 mg.	—	N	9.0 ± 0.24	20.9 (18.5 — 23.3)	6
				−2.4(−5.3 — 0.5)	6*
	Dibenamine 0.1 mg.	N	9.3 ± 0.21	−1.1(−3.0 — 0.8)	9*

Irgapyrin 1 mg.	—	N	8.9 ± 0.24	13.9 (12.6 — 15.2)	12
	—	A	9.3 ± 0.25	12.0 (9.8 — 14.2)	6
				−3.8(−6.3 —−1.3)	6*
	Cortisone 0.1 mg. ×2	A	8.6 ± 0.24	13.4 (11.7 — 15.1)	12
	Epinephrine 1 μg.	A	9.2 ± 0.22	13.0 (11.7 — 14.3)	12
Irgapyrin 0.5 mg.	—	N	9.0 ± 0.25	5.3 (4.5 — 6.1)	12
	—	A	8.8 ± 0.19	5.2 (4.6 — 5.8)	12

N: Normal intact mice
S: Sham-operated mice
A: Adrenalectomized mice
M. R. T.: Mean reaction time
Figures marked with * in the right end column denote number of mice which responded with shortening of the reaction time (early jumping). Maximum shortenings of M. R. T. of these mice are presented with minus signs on the corresponding lines of the adjacent column.

time, both in the normal and the adrenalectomized mice. This observation is quite important since the action of epinephrine in recovering the reduced morphine analgesia in adrenalectomized animals is not indicative of the analgesic action inherent in epinephrine itself. Some of these results are illustrated in Fig. 1.

In normal mice administered with subcutaneous injection of 0.5 mg./10 g. of tetraethylammonium bromide at the same time with morphine, the effect of morphine was reduced. The effect of morphine was more effectively suppressed in normal mice injected with 0.05 mg./10 g. of priscol and 0.1 mg./10 g. of dibenamine, 1 hour before. These two drugs also caused a slight reduction of morphine analgesia in adrenalectomized mice.

Meperidine. The increase of reaction time by the subcutaneous injection of 0.2 mg./10 g. of meperidine hydrochloride in normal intact and sham-operated mice reached the maximum 30 minutes after the injection but the maximum increase was markedly reduced in the adrenalectomized mice, the maximum being reached 15 minutes later than the two former groups. In the latter group, the concurrent use of 1 μg./10 g. of epinephrine with meperidine produced an effect approximating that of the mice in the two former groups. Injection of 0.1 mg./10 g. of dibenamine in normal intact mice, 1 hour before meperidine, effected significant reduction of the maximum increase of the mean reaction time by the latter. These findings were all similar to those of morphine.

Ohton. The effect of 0.08 mg./10 g. of ohton in normal intact mice was comparable to that of 0.1 mg./10 g. of morphine. However, the effect of ohton was different from that of morphine or meperidine in that the

course of lengthening of the reaction time (maximum after 30 minutes) was entirely the same in adrenalectomized and normal intact mice. The effect of ohton in normal intact mice was not modified by 0.5 mg./10 g. of tetraethylammonium bromide or 0.1 mg./10 g. of dibenamine administered 1 hour before.

Aminopyrine. In normal intact mice the injection of 1 mg./10 g. of aminopyrine effected an increase of reaction time, the maximum increase (mean, 11.7 sec.) being observed 30 miuntes after the injection and gradual

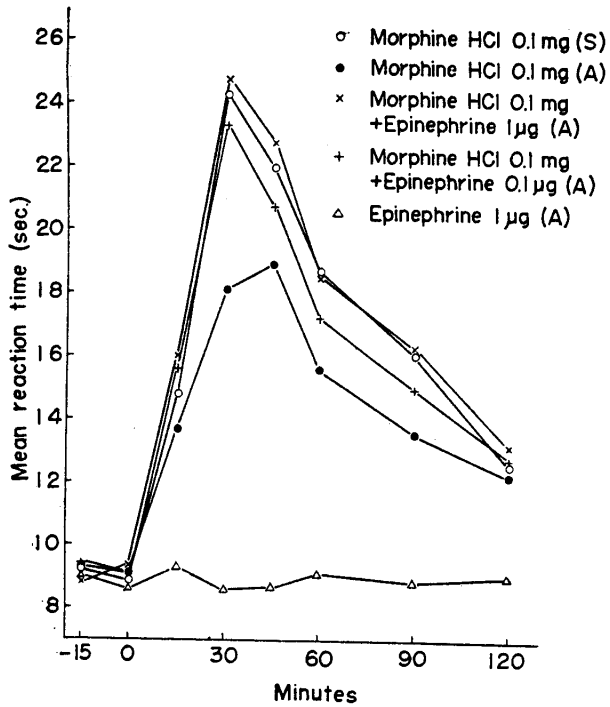

Fig. 1. Effect of epinephrine on the reduced analgesic effect (reduced prolongation of the mean reaction time on the hot-plate) of morphine in the adrenalectomized mice. A: adrenalectomized, S: sham-operated mice. Doses are in terms of per 10 g. body weight.

recovery to the preinjection reaction threshold over the next 2 hours. In the majority of the adrenalectomized mice receiving the same dose, however, a peculiar phenomenon occurred; instead of prologation, the reaction time was shortened. These animals, soon after being placed on the hot plate, started to jump about and attempted to jump out of the restraining glass cylinder. The reduction of reaction time by this jumping was most marked during 30—60 minutes after the injection. Even in the

adrenalectomized mice, a small number of which did not exhibit such jumping movements, all showed an increase of the mean reaction time, the same as in the normal intact mice. Therefore, calculation of the mean value of maximal changes in reaction time was made separately for the two kinds of mice (Fig. 2 and Table 1).

The group of adrenalectomized mice previously given cortisone or the group given 1 μg./10 g. of epinephrine at the same time, failed to show early jumping movements with 1 mg./10 g. of aminopyrine and all the mice showed an increase of reaction time approximately the same as the normal intact mice.

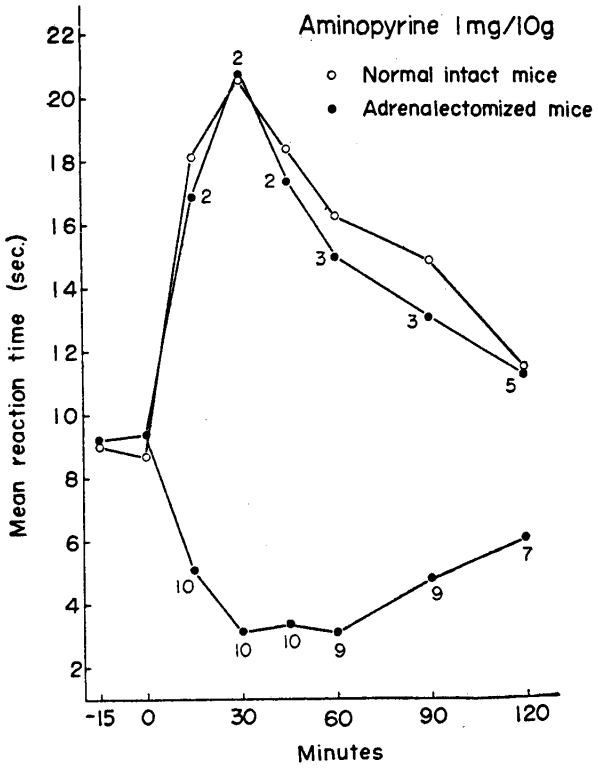

Fig. 2. Splitted responses of mice to aminopyrine after adrenalectomy. Figures denote the number of responses among 12 mice to a group.

When 1 mg./10 g. of aminopyrine is administered to normal intact mice 1 hour after the injection of 0.1 mg./10 g. of dibenamine, about 1/3 of the group of mice exhibit the early jumping movement for 15—60 minutes. However, in the other mice, there has been no effect of dibenamine on the increase of reaction time. With a larger dose, 2.5 mg./10 g.,

aminopyrine causes one-half of the normal intact mice to exhibit the early jumping movements 15—60 minutes after the injection. If 0.1 mg. /10 g. of dibenamine is given 1 hour earlier, 1/3 of the normal intact mice die within 1 hour after the administration of 2.5 mg./10 g. of aminopyrine and the majority of surviving mice exhibit early jumping for a long period of 15—120 minutes after the injection.

Irgapyrin. The effect of irgapyrin in prolonging the reaction time in normal intact mice was slightly greater than that of aminopyrine. Also with this preparation, the adrenalectomized mice reacted in two different ways by the injection of 1 mg./10 g. those showing an early jumping movement and those showing an increase of mean reaction time appearing as in the normal intact mice. In this case, however, the jumping response was found only in about one-half of the group, smaller than in the case of aminopyrine. Both cortisone and epinephrine prevented the appearance of such jumping response in mice, as in the case of aminopyrine. At 0.5 mg./10 g. of irgapyrin, jumping movement was not observed even in adrenalectomized mice and only a prolongation of pain reaction time occurred, as in normal intact mice.

DISCUSSION

In the present experiments, the analgesic effects of morphine have been clearly observed to decrease in adrenalectomized mice, indicating that the adrenal glands play an important rôle in such an effect of morphine. The experiments also indicate that this decrease of morphine effect has been completely restored by the concurrent use of 100 μg./kg. of epinephrine and markedly by 10 μg./kg. Not only the sympatholytic agents, priscol and dibenamine, but also tetraethylammonium salt which blocks the release of the medullary epinephrine[11], weaken the analgesic effects of morphine in the normal intact mice. From these data, it seems reasonable to assume that the analgesic effect of morphine is normally supplemented by epinephrine apparently originating in the adrenal medulla.

However, as far the amount of epinephrine used in the present experiments, it is clear that the administration of epinephrine alone do not show any analgesic effect, either in the normal intact or the adrenalectomized mice. Furthermore, epinephrine has been found to cause only an insignificant increase of the effect of morphine in the sham-operated mice. This fact indicated that the action of epinephrine in normalizing the reduced analgesic effect of morphine in the adrenalectomized mice should not be hastily assumed as an additive increase of effect, as has

been interpreted by the early proponents of the epinephrine hypothesis. The question then is by what mechanism epinephrine increases the morphine effect in the adrenalectomized animals.

It has already been pointed out that morphine exerts a dual effect of a stimulant and a depressant at all the levels of integration in the cerebrospinal axis. Combination of such actions in morphine and related compounds is especially prominent in the spinal cord. A part of the effects of narcotics in the prolongation of reaction time of rat's tail flick and dog's cutaneous maximus twitch in response to radiant heat stimuli is assumed to be a measure of the depressant effect on the spinal reflex[13,14]. A small amount of morphine chiefly depresses polysynaptic reflex discharges[15]. However, TAKAGI *et al.*[16], who made detailed examination of the action of morphine on the spinal reflex, have demonstrated that its depressant effect was apparent in normal intact or midbrain animals but was transitory in the spinal animal. In their experiments based on the destruction of reticular formation of the brain stem, they assumed that this depressant effect is due to a more powerful stimulating action of morphine on the inhibitory region rather than the fascilatory region of the brain stem and cervical reticular formation. YANAI[17] and TAKAGI *et al.*[16] observed that a small amount of epinephrine, though in itself being unable to affect the spinal reflex, supresses it in synergy with morphine; and since this synergy is not observed in the case of thalamic, midbrain, and spinal animals, they concluded that cerebral cortex is in some way associated with this action of epinephrine. On the other hand, there are experimental results suggesting that epinephrine acts on certain centers of the brain stem to induce analgesia and sleep[7,8]. In any case, if there is such an action in epinephrine that affects these higher centers, an exacerbation of depressant effect of morphine on the spinal reflex in the adrenalectomized animals may be caused by a small amount of epinephrine insufficient to cause any analgesic effect by itself. Further, a part of depressant effects of morphine on the spinal reflex in the intact animal may be measureably potentiated by epinephrine released from the adrenal medulla by the narcotics.

MILLER *et al.*[9] observed the hyperglycemic effect in a dog given a subcutaneous injection of 5 mg./kg. of morphine hydrochloride, but the same phenomenon was found to occur in a rat only after 40 mg./kg. of the narcotic. SATO and OHMI[5] showed that the rate of epinephrine release from the adrenal veins was 0.07—0.58 μg./kg./min. after a subcutaneous injection of 10—40 mg./kg. of morphine in a dog. It follows that the release of epinephrine from the adrenal medulla may be quite small in a

mouse given 10 mg./kg. of morphine in the present experiments. Even if the amount is small, a possibility of epinephrine to potentiate the effect of morphine of spinal reflex suppression, however, cannot be denied, as is clear from the work of SCHAYER[18] in tracing the subcutaneously injected β-C^{14}-*dl*-epinephrine in rat organs showed that, in contrast to a high radioactivity at the site of injection, relatively low concentrations were found in the blood and various organs and the counts of the extract of brain were very low or no trace at all. The dosage of epinephrine administered in the present experiments was 1/300 to 1/500 of that used by SCHAYER[18], so that the amount acting on the brain must have been extremely small.

Results obtained from meperidine were similar to those of morphine but no such relationship with the adrenal glands was recognized in the action of ohton. According to TAKAGI *et al.*[16] ohton depresses mono- and polysynaptic spinal reflexes even in low-spinal animals, differing from morphine. YAMAMOTO[19] reported that the effect of ohton on the central neurons of pain afferents was different from that of morphine and shows a strong suppression of reticular formation and the thalamic reticular system, similarly to barbiturates. These facts are all in favor of supporting the newer epinephrine hypothesis of the present author regarding morphine analgesia.

The observations in the present experiments that the central excitation or reflex acceleration of aminopyrine and irgapyrin is increased by the removal of the adrenal glands and is counteracted by the injection of epinephrine seem to support the author's interpretation of the mode of action of epinephrine on the action of morphine. However, it differs in the case of the pyrazolone derivatives in that the enhancement of the jumping reflex is independent of the effect of drugs against the reflex pattern of the pain reaction and that it is suppressed not only by epinephrine but also by cortisone.

SUMMARY

The analgesic effects of morphine and some related compounds, such as meperidine, observed by the conventional method, are supplemented by the release of epinephrine from the adrenal medulla. It is assumed that this action of epinephrine is not due to an additive synergy in the analgesic effect but to the fact that the action of epinephrine on a definite higher center or centers effects synergistically in the reflex depressant action of these analgesic agants. This assumption is based on the following evidences.

Prolongation of reaction time in mice by morphine and meperidine (but not by ohton), determined by the hot-plate method, was significantly reduced by adrenalectomy and this reduction was normalized by the concurrent use of epinephrine, in a small dose which in itself cannot prolong the reaction time. No such action was found in cortisone and DOCA. The effects of morphine and meperidine in prolonging the reaction time were reduced by priscol and dibenamine, as well as by tetraethylammonium salt.

A large dose of pyrazolone derivatives causes, not the prolongation of reaction time but a jumping reflex response in the early stages, indicating central excitation, in part of the mice. The ratio of mice exhibiting such an early reflex increases with adrenalectomy or the administration of dibenamine, and is markedly decreased by epinephrine, insufficient to show any analgesic response by itself, and by cortisone. This action of cortisone indicates some difference in the natures of central excitation by pyrazolones and by morphine.

Judging from the work of SCHAYER[18], the distribution in the brain of epinephrine injected in the dose to normalize the reduced effect of morphine in the adrenalectomized mice, may also be anticipated by the epinephrine which might be released from the adrenal medulla by morphine in an amount much smaller than the "near-lethal doses[9]".

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