ON THE PRESSOR ACTIVITY AND STABILITY OF DIFFERENT MIXTURES OF EPHEDRINE AND PITUITARY (POSTERIOR LOBE) EXTRACT

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In previous work reported from this laboratory (1 and 2), it was shown that (a) ephedrine augments the pressor response to small doses of pituitary extract, (b) ephedrine diminishes or abolishes the depressor response and deleterious cardiac effects of large doses of the extract, and (c) a mixture of ephedrine and pituitary extract is more effective than twice the quantities of the individual agents in restoring the blood pressure, respiration, and general collapse in dogs “shocked” by prolonged intravenous injection of histamine.

On the basis of these observations, Bourne (3), in 1939, tested the effects of a mixture of ½ grain of ephedrine and 5 units of pressor pituitary extract in 86 cases showing varying degrees of circulatory failure following spinal anaesthesia. This group of cases comprised 23 thoracic and 63 abdominal operations. Over the same period of time 113 similar types of cases were also observed, in which spinal anaesthesia was employed, but for which no analeptic was needed. The frequency, therefore, of circulatory failure in spinal anaesthesia, judging from this series of cases, is quite high (43.2 per cent). No detailed comparison of the two groups of cases was given by the writer; however, a number of anaesthesia case records reproduced shows clearly that following spontaneous or intravenous injections of such a mixture (after the systolic pressure had fallen as low as 50 to 60 mm. of mercury) there was a prompt and prolonged increase in the blood pressure level. Bourne recommended the use of this mixture as a valuable analeptic, but employed it only in those cases in which the blood pressure falls to a critical level.

More recently, Bourne, Leigh, Inglis and Howell (4) reported a further series of 282 major thoracic operations performed under spinal anaesthesia in which a similar mixture of the two drugs was administered on 100 additional occasions. In each of these instances again, the mixture was employed only as a last resort when the blood pressure had fallen considerably, and recovery in such cases was all the more striking. It should be noted that in all of these cases the patients received oxygen intranasally and a glucose-saline infusion was maintained throughout the course of the operation and after, as necessary.

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Since ephedrine alone has been used for a long time as an analeptic in connection with spinal anaesthesia, the question arises as to whether the combination of this agent with pressor pituitary extract offers any important therapeutic advantages. Chaikoff (5) has tested this point in a group of 119 cases, including major surgical operations in the upper and lower abdomen, thorax and perineum, in which nupercaine spinal anaesthesia was employed. Thus, it was observed that in 45 patients injected, intramuscularly, eight to ten minutes before the spinal puncture, with ephedrine alone (75 mg.), 17 patients or 37.7 per cent of cases showed "a severe drop in blood pressure," while in a similar group of 36 patients injected similarly with a mixture of ephedrine (40 mg.) and pitressin (5 units), 8 patients or 22.2 per cent of cases showed "a severe drop in blood pressure." Furthermore, in a third group of 38 patients who received a second injection of the mixture just before the incision (provided the blood pressure showed any tendency to fall) only 3 patients or 7.8 per cent of cases showed "any severe fall in blood pressure."

In connection with these results, Chaikoff also stated that following injection of the mixture there was always definite improvement in the general condition of the patient in respect to colour, pulse rate and respiration. Furthermore, in another group of 13 cases in which saline or glucose-saline solution was injected intravenously during the course of the operation after previous administration of the mixture, 70 per cent showed the most satisfactory blood pressure curves throughout the entire operation. It may be concluded, therefore, that this mixture of ephedrine and pituitary extract is more effective than ephedrine alone in the prevention of circulatory failure following spinal anaesthesia.

In view of these encouraging reports it appeared desirable to investigate the relative pressor efficiency of a number of different mixtures of the two agents, since the ratio of the substances employed in previous studies was more or less arbitrarily chosen. Furthermore, as in previous studies the mixtures employed were always freshly made up just before injection, it was desirable to obtain information regarding the stability of such mixtures. The purpose of this communication is to present some results which have been obtained in connection with these questions.

Procedures

This investigation was started in April 1943, when three batches of ampoules ("A," "B" and "C") containing different ratios of ephedrine and pressor pituitary extract were made up. The pressor pituitary preparation employed throughout was Stehle's postlobin-V, as described elsewhere (6). A batch of a fourth mixture ("D") of the two agents was made up in June 1943. The ratio of the two drugs in each of these mixtures was as follows:
(1) Combination “A” contained 24 mg. of ephedrine sulfate and 5 units of postlobin-V, per cubic centimeter, or a ratio of 4.8 mg. of ephedrine per unit of extract.

(2) Combination “B” contained 24 mg. of ephedrine sulfate and 10 units of postlobin-V per cubic centimeter, or a ratio of 2.4 mg. of ephedrine per unit of extract.

(3) Combination “C” contained 48 mg. of ephedrine sulfate and 5 units of postlobin-V per cubic centimeter, or a ratio of 9.6 mg. of ephedrine per unit of extract.

(4) Combination “D” contained 10 mg. of ephedrine sulfate and 20 units of postlobin-V per cubic centimeter, or a ratio of 0.5 mg. of ephedrine per unit of extract.

All the combinations were made up in distilled water, slightly acidified to Congo red paper, ampouled and sterilized at 100 C. for twenty minutes on three successive days. The mixtures were then kept in the refrigerator for use as necessary.

From time to time during a period of nearly a year, these combinations were assayed for their pressor activities in comparison with freshly prepared similar mixtures of the two agents.

Dogs anaesthetized with chloroform were used, and the blood pressure recorded directly from a carotid artery. The animals were also given heparin, and all injections made into an exposed femoral vein.

Comparative Effects of Small Doses of Mixtures

In figure 1 are shown examples of two experiments in which combination “D,” containing a ratio of 0.5 mg. of ephedrine to 1 unit of postlobin-V, was tested. It may be seen from the first sections of the tracings (A, B, C and D) that the pressor response to a dose of 2 units of postlobin-V (A) was not significantly augmented when mixed with 1 mg. of ephedrine. Thus, the responses to 2 units of the extract alone (A), 2 units of the extract after previous administration of 1 mg. of ephedrine (C) and 2 units of the extract mixed with 1 mg. of ephedrine (D) are not much different in intensity, but apparently more prolonged in the two latter instances. At E and F, however, are shown, respectively, the response to 4 units of the extract and 2 mg. of ephedrine from a seven-month old mixture and the response to a similar combination of the two agents when freshly mixed. As can be seen, there is quite close agreement between the two responses. The maximum blood pressure level attained in each instance was only approximately 125 mm. of mercury, despite the relatively large dose of the extract. There was also no evidence of any tachyphylaxis with this combination. In experiment 1B are shown again the relatively poor pressor effects of equal small doses of an eight-month old mixture (B) and a freshly prepared mixture (C).
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Fig. 1. Exp. 1A. Dec. 3, 1943.


A—2 units postlobin-V.
B—1 mg. ephedrine followed at C by 2 units postlobin-V.
D—1 mg. ephedrine freshly mixed with 2 units postlobin-V.
E—0.2 cc. combination "D" ampouled June 7, 1943, containing 2 mg. ephedrine and 4 units postlobin-V.
F—2 mg. ephedrine freshly mixed with 4 units postlobin-V.

An interval of 20 minutes elapsed between injections.

Exp. 1B. Jan. 20, 1944.


A—2 units postlobin-V.
B—0.1 cc. combination "D" ampouled June 7, 1943, containing 1 mg. ephedrine and 2 units postlobin-V.
C—1 mg. ephedrine freshly mixed with 2 units postlobin-V.

An interval of 30 minutes elapsed between injections.
When the ratio of ephedrine to pituitary extract was increased so that there were 2.4 mg. of ephedrine to 1 unit of the extract, the responses obtained were somewhat better. Thus, in figure 2 are shown results of two experiments in which such a combination ("B") was tested. At A, in each experiment, is shown the pressor response to 2 units of postlobin-V alone. In experiment 2A this injection was repeated at C, thirty minutes after the first administration of the extract, and shortly after an injection of 4.8 mg. of ephedrine. The maximum blood pressure level attained was approximately 150 mm. of mercury. This response was also quite prolonged. After a further interval of thirty minutes at D, a similar dose of a fresh mixture of the two agents was injected, and this was repeated at E with an eight-month old preparation, i.e., a mixture ampouled eight months earlier. These two latter responses were not much different, indicating neither the development of tachyphylaxis nor the loss of any activity over such a period of time. Experiment 2B shows similar results, when a ten-month old mixture and a freshly prepared mixture in equal doses were injected, respectively, at B and C. Again, the two responses agree quite well. It may be concluded, therefore, that with the doses of 4.8 mg. of ephedrine and 2 units of extract there was no evidence of lessening in the blood pressure response on repeated administration at thirty-minute intervals, nor any loss of activity in the mixture as long as ten months after preparation.

When the dose of ephedrine mixed with 1 unit of the extract was further increased to 4.8 mg. the summative effects of the mixture were even more striking. Thus, in figure 3 are shown results of two experiments using this combination ("A"). The initial responses to 2 units of postlobin-V alone are shown at A in each experiment. In experiment 3A, at B, can be seen the initial effect of a dose of 9.6 mg. of ephedrine alone, and despite the intense pressor response (200 mg. of mercury) when a dose of 2 units of the extract was superimposed (C), there was a further increase in the blood pressure level. Forty minutes later at D, the blood pressure level was still being maintained at approximately 125 mm. of mercury. The injection of a similar dose of a fresh mixture of the two agents in the same proportion then led to a marked rise in pressure (250 mm. of mercury maximum). After a further interval of forty minutes at E, the blood pressure level was again still quite high, when a similar dose of an eight-month old mixture was injected. As can be seen, although the blood pressure again rose to 200 mm. of mercury, this response was not as good as the preceding one. In experiment 3B, however, when a similar dose of a nine-month old mixture was first injected as shown at B, the pressor response again was better than that obtained with a similar fresh mixture of the same ratio, as shown at C. It must be concluded, therefore, that there was no loss of activity in the preparation, but that apparently owing to the high ephedrine dose there was evidence of tachy-
Fig. 2. Exp. 2A. Dec. 6, 1943.


A—2 units postlobin-V.
B—4.8 mg. ephedrine followed at C by 2 units postlobin-V.
D—4.8 mg. ephedrine freshly mixed with 2 units postlobin-V.
E—0.2 cc. combination "B" ampouled April 25, 1943, containing 4.8 mg. ephedrine and
2 units postlobin-V.

An interval of 30 minutes elapsed between injections.

Exp. 2B. Feb. 10, 1944.


A—2 units postlobin-V.
B—0.2 cc. combination "B" ampouled April 26, 1943, containing 4.8 mg. ephedrine and
2 units postlobin-V.
C—4.8 mg. ephedrine freshly mixed with 2 units postlobin-V.

An interval of 30 minutes elapsed between injections.
Fig. 3. Exp. 3A. Dec. 7, 1943.


A—2 units postlobin-V.
B—0.6 mg. ephedrine followed at C by 2 units postlobin-V.
D—0.6 mg. ephedrine freshly mixed with 2 units postlobin-V.
E—0.4 cc. combination "A" ampouled April 26, 1943, containing 0.6 mg. ephedrine and 2 units postlobin-V.

An interval of 40 minutes elapsed between injections.
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Phylaxis. It might be added further that despite this fact the highest blood pressure levels in all of the experiments performed were generally obtained with this combination.

When the ratio of ephedrine in the mixture was further increased so that 1 unit of the extract was mixed with 9.6 mg of ephedrine (combination "C"), the responses obtained were in general less satisfactory. Thus in figure 4 are shown some results obtained with combination "C." Again, in each experiment at A the response to 2 units of the extract alone is seen. At B, in experiment 4A, 19.2 mg of ephedrine alone was injected, followed shortly at C, by 2 units of postlobin-V. The maximum blood pressure rise was approximately 150 mm of mercury, and the blood pressure level was higher than in the control period as long as forty minutes later, as shown at D. At this time a similar fresh mixture of the two agents was injected, and after a further period of forty minutes at E, a similar dose of a six-month old mixture. Both of these latter responses agreed fairly well, but they were much smaller than the initial response of the two drugs (B and C). In experiment 4B, furthermore, when a similar dose of a nine-month old mixture was injected at B, the pressor response was much better than that resulting from a similar fresh mixture forty minutes later. It may be concluded, therefore, that there was again no loss of activity in this mixture, but that such a combination showed a still greater degree of tachyphylaxis. In general, this combination "C" was less effective than combination "A" despite the higher ratio of ephedrine.

Effects of Large Doses of Mixtures

In figures 5 and 6 are shown the effects of large doses of freshly prepared mixtures of ephedrine and postlobin-V in varying ratios, and with repeated injections at thirty-minute intervals for three successive administrations.

Since, as is well known, injection of a large dose of pituitary extract leads to a mixed pressor-depressor response, it was of interest to ascertain the minimal amount of ephedrine that would abolish this depressor response. In figure 5, experiment 12, it may be seen that an initial injection of 5 mg of ephedrine with 20 units of the extract or a ratio of 0.25 mg of ephedrine to 1 unit of extract (A) leads to almost complete abolition of the depressor response (there is only a slight notch in the blood pressure curve). When the dose of ephedrine was increased to

Exp. 3B. Jan. 18, 1941.


A—2 units postlobin-V.
B—0.4 cc. combination "A" ampouled April 26, 1943, containing 9.6 mg ephedrine and 2 units postlobin-V.
C—9.6 mg ephedrine freshly mixed with 2 units postlobin-V.

An interval of 40 minutes elapsed between injections.
Fig. 4. Exp. 4A. Dec. 7, 1943.


A—2 units postlobin-V.
B—19.2 mg. ephedrine followed at C by 2 units postlobin-V.
D—19.2 mg. ephedrine freshly mixed with 2 units postlobin-V.
E—0.4 cc. combination "C" ampouled April 25, 1943, containing 19.2 mg. ephedrine and 2 units postlobin-V.

An interval of 40 minutes elapsed between injections.
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10 mg., however, and the mixture with 20 units of the extract (a ratio of 0.5 mg. of ephedrine to 1 unit of extract) injected as shown in experiment 13 (A) there was no evidence of any depressor response or deleterious cardiac effect from 20 units of the extract.

When higher ratios of ephedrine were employed in conjunction with the extract, it is of interest to note the effects. Thus, in the 3 experiments shown in figure 6, a dose of 48 mg. of ephedrine was injected after being mixed, respectively, with 5 units (a ratio of 9.6 mg. of ephedrine to 1 unit of extract), with 10 units (a ratio of 4.8 mg. of ephedrine to 1 unit of extract) and with 20 units (a ratio of 2.4 mg. to 1 unit of extract) of pituitary extract. In experiment 5, the initial rise in blood pressure (Δ) was less than in experiment 6(Δ), while in experiment 7(Δ) during the course of the rise in blood pressure there were marked fluctuations in the blood pressure curve, indicating a deleterious effect on the heart. Repetitions of these injections as shown at B and C, in experiments 5 and 7 led to strikingly less and less responses, while in experiment 6, despite the fact that forty minutes after the injections the blood pressure was being maintained at a higher level than in the other experiments, repetitions of the injection at B and C led to quite good responses. In fact, the blood pressure rose to somewhat more than 200 mm. of mercury after each injection in this experiment, and was well maintained for several minutes.

Finally, comparing the results obtained in experiment 7 (fig. 6) with those shown in experiments 12 and 13 (fig. 5), it is clear that the injection of a combination of a large dose of ephedrine with a large dose of pituitary extract leads to definite evidence of circulatory damage, while it requires only small amounts of ephedrine to offset the deleterious cardiac effect of pituitary extract. These findings are in agreement with previously described results (1) obtained with the heart-lung preparation, in which it was observed that although a dose of 2.5 mg. of ephedrine could completely abolish the deleterious cardiac effects of a dose of 10 mg. of a laboratory pituitary preparation employed at that time, such a dose of the extract when mixed with 10 mg. of ephedrine (although itself without obvious deleterious effects upon the heart) leads rapidly to a fatal outcome. It is thus obvious that a large dose of ephedrine renders the heart more susceptible than normal to the deleterious action of pituitary extract. The mechanism of this phenomenon is not clear.

Exp. 4B. Jan. 12, 1944.


A—2 units postobin-V.
B—0.4 cc. combination "C" ampouled April 26, 1943, containing 19.2 mg. ephedrine with 2 units postobin-V.
C—19.2 mg. ephedrine freshly mixed with 2 units postobin-V.

An interval of 40 minutes elapsed between injections.
Fig. 5. *Exp. 12.*

A, B, and C—5 mg. ephedrine freshly mixed with 20 units postlobin-V.
An interval of 30 minutes elapsed between injections.

*Exp. 13.*

A, B, and C—10 mg. ephedrine freshly mixed with 20 units postlobin-V.
An interval of 30 minutes elapsed between injections.
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Discussion

The above described experiments show that when ephedrine is mixed with pituitary extract for injection it is important to consider the ratio of the two agents employed. Thus, comparative pressor responses to small doses of such mixtures show that when 2 units of the extract are mixed with 1 mg., 4.8 mg., 9.6 mg., and 19.2 mg., respectively, of ephedrine, the most intense pressor response is observed, in general, with the preparation containing 9.6 mg. When repeated at forty-minute intervals, however, there is some evidence of a lessened response with the combination. On the other hand, the pressor responses observed with the preparation containing 19.2 mg. were in general less marked. These responses were also no greater than were observed with the preparation containing only 4.8 mg. of ephedrine. With the former combination, however, tachyphylaxis appeared after the first injection, while with the latter there was little or no evidence of tachyphylaxis even with 2 or 3 injections made at thirty-minute intervals. The preparation containing 1 mg. of ephedrine showed the least pressor activity in similar small doses, that is, when employed with 2 units of the extract.

It is also shown that it requires only a relatively small quantity of ephedrine (10 mg.) to counteract the deleterious cardiac effects of a large dose of pituitary extract (20 units) in the dog under chloroform anesthesia. Furthermore, such a combination, although the least effective in small doses, in large doses raises the blood pressure effectively, and may be repeated with good effects at thirty-minute intervals. On the other hand, the same dose of pituitary extract (20 units) when mixed with a large dose of ephedrine (48 mg.) exerts a pronounced deleterious effect upon the circulation, and leads only to a relatively slight pressor effect on repetition. The former combination is therefore safe, the latter dangerous.

Of the two intermediate mixtures studied, namely, the one containing 5 units of extract with 48 mg. of ephedrine, and the one containing 10 units of extract with 48 mg. of ephedrine, the former was the less effective on initial administration and also led to poorer responses on repetition. The latter combination, therefore, appears to be most effective on initial injection in large doses and a good response was observed on second and third administrations at thirty-minute intervals.

Since the pressor responses to small doses of this combination were also the most pronounced, it may be concluded that a ratio of 4.8 mg. of ephedrine to 1 unit of pituitary extract appears to be the optimal combination in which the two agents should be used.

Furthermore, while such a combination can be readily made up freshly by mixing these ratios of the two agents, it has been shown that such a mixture after ampouling and sterilizing still retains its activity as long as from seven to eleven months, if kept in a refrigerator.
Fig. 6. Exp. 5.

A, B, and C—48 mg. ephedrine freshly mixed with 5 units postlobin-V.
An interval of 30 minutes elapsed between injections.

Exp. 6.

A, B, and C—48 mg. ephedrine freshly mixed with 10 units postlobin-V.
An interval of 30 minutes elapsed between injections.
Summary

Four ampouled mixtures containing different ratios of ephedrine and pressor pituitary extract (postlobin-V) have been tested for their comparative effects upon the blood pressure of the chloretonized dog, in small and large doses.

It is shown that the deleterious cardiac effect of a large dose of pituitary extract can be completely abolished by relatively small doses of ephedrine. However, the most effective combination, in respect to pressor activity and effect of repeated doses, appears to be a mixture containing a ratio of 4.8 mg. of ephedrine to 1 unit of pressor pituitary extract.

It is also shown that large doses of the extract mixed with the large doses of ephedrine lead to marked deleterious circulatory effects.

Finally, the ampouled and sterilized mixtures in the various proportions studied show no significant loss of activity as long as seven to eleven months after preparation, when kept in the refrigerator.

References


Exp. 7.

A, B, and C—48 mg. ephedrine freshly mixed with 20 units postlobin-V.
An interval of 30 minutes elapsed between injections.