THE INFLUENCE OF CERTAIN CHEMICAL SUBSTANCES ON THE INITIATION OF SENSORY DISCHARGES IN PULMONARY AND GASTRIC STRETCH RECEPTORS AND ATRIAL RECEPTORS

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In recent years there have been many investigations designed to elucidate the mechanism of excitation of cutaneous mechanoreceptors and carotid baroreceptors by chemical substances (Brown & Gray, 1948; Habgood, 1950; Jarisch, Landgren, Neil & Zotterman, 1952; Landgren, Neil & Zotterman, 1952; Witzleb, 1952; Liljestrand, 1953; Douglas & Gray, 1953; Landgren, Skouby & Zotterman, 1953; Gollwitzer-Meier & Witzleb, 1954; Jarret, 1955; Diamond, 1955; Robertson, Swan & Whitteridge, 1956; Lowenstein, 1956). Unlike chemoreceptors these receptors are normally activated by mechanical stimuli. Several different types of receptors normally also excited by mechanical stimuli are now known to exist in the thoracic and abdominal viscera, and the responses of these to chemical substances have also been studied. Of these, the pulmonary stretch receptors have been most frequently studied by means of chemical substances (Whitteridge & Bülbbring, 1944; Walsh, 1947; Whitteridge, 1948; Meier, Bein & Helmich, 1949; Dawes, Mott & Widdicombe, 1951a, b; Mott & Paintal, 1953; Schneider & Yonkman, 1953; Widdicombe, 1954). The cardiac receptors have also been studied in this way (Amann & Schaefer, 1943; Jarisch & Zotterman, 1948; Paintal, 1953b, 1955b). In addition, the responses of two recently encountered sensory systems, the gastric stretch receptors and the pulmonary deflation receptors, to several diverse chemical substances have also been described (Paintal, 1954b, 1955a).

However, in spite of the considerable amount of information now available, little is known about the mechanism of excitation of these mechanoreceptors by different chemical substances. This is not surprising, because as far as the visceral receptors are concerned, interest has been centred on the reflex

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effects produced by the chemical substances and not on the actual mechanism of excitation of the sensory receptors.

One group of substances known to stimulate different types of visceral sensory receptors is veratrum and its individual alkaloids (Amann & Schaefer, 1943; Jarisch & Zotterman, 1948; Meier et al. 1949; Dawes et al. 1951a; Paintal, 1955b), but apparently little is known about the mechanism of stimulation of these receptors by the alkaloids of veratrum. This is surprising, as a great deal of work has been done on the effects of veratrum on peripheral nerves and a large amount of electro-physiological information is available on the changes in nerve following the application of veratrum (see review by Krayer & Acheson, 1946). Indeed, it is noteworthy that many investigators working with veratrum or its alkaloids have failed to notice the remarkable phenomenon of intense stimulation coupled with desensitization of the receptors produced by the alkaloids of veratrum. This response contrasts with the pure sensitization of pulmonary stretch receptors by volatile anaesthetics first described by Whitteridge & Bülbring (1944). A similar sensitization has also been seen in carotid baroreceptors following volatile anaesthetics (Robertson et al. 1956).

In view of the above it was felt that much more information was needed and the present investigation was undertaken in an attempt to collect information which will throw some light on the mechanism of stimulation of receptors by veratrum alkaloids. The following types of sensory receptors normally aroused only by mechanical stimuli have been studied: pulmonary stretch receptors (Adrian, 1933), gastric stretch receptors (Paintal, 1954a), regarded by Iggo (1955) as tension receptors, and atrial receptors type A and type B (Paintal, 1953a).

METHODS

Experiments were carried out on adult cats. The details of the techniques used for the dissection of vagal nerve fibres were identical with those used earlier (Paintal, 1953a). The methods of identifying the atrial receptors and the gastric stretch receptors were similar to those reported in previous papers (Paintal, 1953a, 1954a).

In studying the responses of the pulmonary stretch receptors, the cat's chest was opened and artificial respiration carried out with a Palmer Ideal respiratory pump. The threshold of excitation of these receptors was determined by inflating the lungs with known volumes of air from a 100 ml. syringe. The same system was used to estimate the adaptation rates of these receptors. In administering trichlorethylene, air from the respiratory pump was blown through a 1 in. column of the anaesthetic contained in a Wolff's bottle. The more desirable system used by Whitteridge & Bülbring (1954) was not considered necessary in the present case. The pump had a linear stroke up to peak inflation. Thereafter the lungs were allowed to collapse rapidly, as illustrated by the intratracheal pressure records in Fig. 1.

The alkaloids of veratrum were injected through catheters either into the right atrium when studying the responses of the pulmonary stretch receptors and atrial receptors or into the abdominal aorta when recording the responses of gastric stretch receptors. The aortic catheter was introduced at the point of bifurcation of the aorta and pushed cephalad so that its tip lay cephalad to the coeliac artery. The atrial catheter was introduced through the external jugular vein. The dead space of these catheters was always taken into account in estimating the amount of the chemical substances introduced into the circulation.
The alkaloids of veratrum injected were germitrine, germerine, neogermitrine (all kindly supplied by the Squibb Institute for Medical Research, New Brunswick) and veratridine (kindly supplied by Professor Otto Krayer). These were first dissolved in dilute HCl and then diluted with 0.9% NaCl (w/v). Veriloid (Riker Laboratories), which is a mixture of several alkaloids of veratrum viride, was diluted with 0.9% NaCl (w/v). The final concentrations of the alkaloids injected varied from 100 to 200 µg/ml.; the volume of injection was kept constant at 2 ml. The injection signal consisted of a 2 V light source connected to a foot switch.

RESULTS

Stimulation and desensitization by veratrum alkaloids

Pulmonary stretch receptors

All the responses of these receptors were studied after opening the chest. Under these conditions and while the lungs are collapsed many of the pulmonary stretch fibres are normally inactive, but after the injection of one or a mixture of veratrum alkaloids they are stimulated; a continuous discharge, which persists during collapse of the lungs, sets in. This characteristic action of certain veratrum alkaloids is well illustrated in Fig. 1A and B which show the response in one fibre to 26 µg germitrine injected into the right atrium. This, and the other alkaloids, germerine, veratridine and neogermitrine used in this investigation, produced similar responses in 22 out of 24 fibres when injected in amounts ranging from 26 to 400 µg; the two fibres that were not stimulated were unaffected by even 350 µg germitrine. The substance used most frequently was germitrine, and 26 µg of this, injected intra-atrially, was effective in stimulating nine pulmonary stretch receptors; but it is felt that lower amounts would also have stimulated these receptors.

The interval between the beginning of injection and the onset of stimulation varied in different fibres from 2.5 sec to 3 min. The onset was gradual or sudden; in the latter case it was usually precipitated by inflation of the lungs. Maximal stimulation was rapidly attained when the onset was sudden (Fig. 2). Once stimulation had set in, the further course of stimulation followed a pattern that was common to many fibres. A typical response is illustrated in Fig. 2, which shows graphically the activity in a pulmonary stretch fibre before and after 26 µg germitrine. In this fibre the stimulation which occurred about 4 sec after the injection of germitrine rapidly gained momentum to reach a resting discharge of over 200 impulses/sec; as the figure shows, this fell during the subsequent few seconds. The fourth inflation after the injection of germitrine terminated the discharge and a very short period of inactivity then followed. More commonly, this period was of much longer duration, and during this period of depression normal inflation of the lungs did not produce the discharge which it had previously done, but a deep rapid inflation sometimes produced a short burst of impulses (Fig. 5). This phase was followed by the sudden appearance of spontaneous discharge of impulses (Fig. 2); sometimes this was precipitated by inflation of the lungs, but often, as in Fig. 2,
Fig. 1. Effect of germitrine on pulmonary stretch receptors in a cat with open chest and artificial ventilation. A shows the normal response in a pulmonary stretch fibre to inflation of the lungs. B begins 8 sec after injection of 26 μg germitrine into the right atrium; the receptor is intensely stimulated and the discharge of impulses persists during collapse of the lungs. C is taken 14 sec after injection of germitrine; note that the frequency of discharge is less than that in B and the discharge gives the appearance of irregularity owing to the dropping out of impulses. D is another fibre which also illustrates the dropping out of impulses and the sudden termination of the discharge. From above downwards in each record: impulses in a pulmonary stretch receptor; time in 1/8 sec; and record of intratracheal pressure which has been retouched in B and C.
inflation had nothing to do with the appearance of this discharge. This phase of activity would then continue until it ceased as suddenly as it appeared. This cycle of events would repeat itself for several minutes until the responses from the receptors eventually ceased altogether.

Fig. 2. Graph showing the course of stimulation of a pulmonary stretch receptor in a cat with open chest and artificial ventilation (interrupted curve). The numbers indicate the rise in frequency of discharge produced by the standard inflation of the lungs. The graph shows clearly that the receptor is desensitized although stimulated intensely. Arrows mark the injection of 26 μg germitrine into the right atrium.

Fig. 3. Graph showing course of stimulation of a pulmonary stretch receptor following a large dose of germitrine (350 μg) at arrow. The chest was opened and the lungs were kept collapsed.

Injection of relatively very large doses of germitrine (about 350 μg) in the absence of natural stimulation, i.e. keeping the lungs collapsed, sometimes produced a response which is illustrated graphically in Fig. 3. In this fibre it seemed that, once stimulation had started, it continued increasingly, to end in a self-produced paralysis since impulses could not be produced in this fibre for some time after the termination of the discharge.
No reduction in the threshold of stimulation by inflation of the lungs was observed in any of the 8 fibres in which changes in the threshold were looked for. In fact, in four fibres, the threshold appeared to have increased, and in 3 fibres it was decidedly increased. Stimulation of the receptors by the alkaloids was not associated with a reduction in the adaptation rate of the receptors (Fig. 4). In most fibres in the later stages the adaptation rate was greatly increased (Fig. 5).

![Graph showing responses of a pulmonary stretch receptor to maintained 100 ml. inflation of the lungs before and after 350 μg germitrine.](image)

The most striking feature of stimulation by the alkaloids was that the receptors were simultaneously desensitized to their natural stimulus (Figs. 2, 4, 10 and 12). An initial phase of sensitization was not seen; desensitization seemed to begin from the moment the receptors were stimulated. In many receptors inflation of the lungs during the period of stimulation increased the frequency of discharge by only a fraction of the increase that occurred before the alkaloids were injected (Fig. 2). Indeed in some receptors although the resting discharge was increased—e.g. as shown in Fig. 4 in which germitrine increased the resting discharge from 30 to 62 impulses/sec—the peak frequency attained during peak inflation of the lungs by 100 ml. air was less than that attained...
before injection of germitrine. This figure, therefore, shows clearly that germitrine had desensitized the receptor to its natural stimulus.

Desensitization is also clearly illustrated in Figs. 2, 10 and 12. In the case of Fig. 10 it might be asserted that the low increase in the frequency of discharge was due to the receptor having reached its limit of repetition, i.e. 160 impulses/sec. This is not the case because this receptor attained a peak frequency of 182 impulses/sec during inflation with the same volume of air after administration of trichlorethylene, which sensitizes these receptors remarkably (Whitteridge & Bulbring, 1944). In most instances in which desensitization was evident it was confirmed that the low rise in the frequency of discharge during inflation was not due to the receptor having reached its limit of response; a much higher frequency could be attained by inflating the lungs with large volumes of air. In the case of the fibre shown in Fig. 4, this procedure was unnecessary because normally the receptor attained a higher frequency of discharge with the standard inflation.

Fatigue of the ending produced by the high frequency continuous discharge is apparently not the cause of this behaviour. This was suggested by the behaviour of some fibres in which a persistent high frequency discharge was produced by keeping the lungs inflated at a certain volume. Superimposition of the standard inflation now produced a much greater increase in the discharge frequency than that observed after injection of the alkaloids. The possibility of non-linear responses occurring at high levels of inflation should, however, be kept in mind.

The pattern of cyclical activity (i.e. periods of discharge of impulses interspersed with periods of complete inactivity) following injection of germitrine varied somewhat in different fibres. Sometimes a discharge cycle could be precipitated by inflation of the lungs and this discharge would then continue long after the inflation was over. In many cases, however, inflation seemed to have nothing to do with the origin of the discharge (Fig 6 A). In one fibre, after 350 µg germitrine, the cyclical activity consisted of a discharge of impulses of 15–35 sec duration interspersed with periods of total depression lasting for about 2 min. In this fibre the onset of the excitatory phase was sudden, resembling the onset in another fibre illustrated in Fig. 6 A. In another fibre the periods of depression lasted about 1 min; in this, the phase of activity started at a low frequency of discharge and increased gradually to attain its peak frequency which lasted for a few seconds, being terminated by inflation of the lungs. This gradual increase resembled that seen by Matthews (1933) in the muscle spindle during occlusion of the blood supply to the muscle. The periods of activity gave the impression that, once the discharge started, its own activity led to its end.

One important point illustrated in Fig. 6 A is that when the discharge started after a silence in some fibres, it did so by starting near its peak
frequency—it seemed as if a clamp had been applied during the silent period and was suddenly released. Fig. 6B, which is a record from another fibre, shows that the termination of the discharge was also as sudden; this was typical of many fibres.

Fig. 5. Increase in the adaptation rate during the silent phase of cyclical activity following 350$\mu$g germitrine. A shows the normal slowly adapting discharge in a pulmonary stretch fibre produced by 40 ml. inflation of the lungs; B, which is a record of the same fibre, shows the very rapidly adapting discharge produced by a large inflation during the silent phase of cyclical activity following 350$\mu$g germitrine. From above downwards in both records: impulses in a fibre; time, $1/10$ sec; and intratracheal pressure.

Fig. 6. Records showing sudden onset, A, and sudden termination, B, of spontaneous discharge in two different pulmonary stretch fibres after germitrine. Note that the discharge in A starts at its peak frequency, and the termination of the cyclical discharge in B also occurs near its peak frequency. In both cases the lungs were collapsed. In A the cyclical activity occurred after 26$\mu$g germitrine; in B after 350$\mu$g. From above downwards in both; impulses in a fibre; time, $1/10$ sec; and intratracheal pressure record (which has been retouched in A).
The period of depression in the cyclical activity was characterized either by total unresponsiveness of the receptor or, if there was a response, by a short burst of impulses which could be evoked by a large rapid inflation of the lungs (Fig. 5). This type of response was also seen during the depression before all responses from the receptor disappeared.

Eventually, after the injection of 200–400$\mu$g germitrine, irreversible depression of the receptor set in, after which no impulses could be aroused in the receptors by maximal inflation. This was clearly present in 11 fibres. Usually this occurred after several minutes of intense activity or after a number of cycles of activity alternating with depression. In one fibre, however, total depression set in after 6 sec of stimulation produced by about 300$\mu$g germitrine.

During the period of stimulation of the pulmonary stretch receptors impulses dropped out in a number of fibres. This usually occurred a short time after the beginning of the stimulation by germitrine, and it gave the appearance of irregularity (Fig. 1, C, D). It is perhaps significant that the irregularity caused by the dropping out of impulses occurred when the level of discharge began to fall. Often, soon after the impulses began dropping out, the discharge ceased for a few seconds. In one case the discharge which started after this began hesitatingly, but soon after it had gained in frequency it became regular. The frequency at which impulses began dropping out was about 70/sec, in fact sometimes over 150/sec, as shown in Fig. 1C. Irregularity of discharge is a feature of receptors firing at a low frequency (Buller, Nicholls & Ström, 1953) and it is also known that increasing the frequency regularizes the discharge; but the frequency at which this occurs is very low when compared with the frequency at which dropping out of impulses was observed in the present experiments.

By inflating the lungs rapidly with large volumes of air, it is possible to produce in many pulmonary stretch receptors a very high frequency discharge (250–350 impulses/sec) which then suddenly falls and soon ceases altogether (Wedensky effect). The frequency at which this happened was examined before and after administering germitrine; in every case this frequency was greatly reduced after germitrine. Indeed, from the many observations (e.g. Fig. 2) in which the continuous discharge was terminated by inflation of the lungs, it is clear that the receptors fail to discharge at markedly low frequencies.

**Atrial receptors**

In an earlier investigation on ventricular pressure receptors and atrial receptors it was shown clearly that veratridine and veriloid stimulated all the ventricular receptors examined and about a third of the left atrial receptors; the right atrial receptors were unaffected by the doses of the substances injected (Paintal, 1955b). However, owing to the absence of a simultaneous record of cardiac pressures and atrial volume, it could not be decided whether the
receptors were sensitized or desensitized by the alkaloids. In fact, not much attention was paid to this aspect of the problem because the main aim at that time was to establish the existence of the ventricular pressure receptors and to study their role and that of the left atrial receptors in the Bezold reflex.

The results obtained in that investigation have now been re-examined in the light of the fact that veratrum alkaloids desensitize the pulmonary stretch receptors; and it seems very likely that the low ratio between peak frequency and lowest frequency obtained during the height of stimulation of the left atrial and ventricular pressure receptors was due to the desensitization of these receptors. In some of the records the marked reduction or absence of a cardiac rhythm during intense stimulation of the receptors strongly suggests that the receptors were rendered less capable of responding to their natural stimulus. This reasoning is valid provided the physiological stimulus—e.g. atrial filling in the case of the left atrial type B receptors—was not reduced. Reduction in atrial volume is unlikely, because slowing of the heart which follows the injection of the alkaloids results in greater filling of the atria. Another point is that in many cases the left atrial receptors were stimulated after an interval of \( \frac{1}{2} \)–1 min, i.e. at a time when the new conditions of lowered blood pressure and slowing of the heart would have temporarily stabilized themselves. The small increase in the peak frequency occasioned by cardiac activity a few seconds after the onset of this stimulation cannot, therefore, be attributed to altered haemodynamic conditions.

Fig. 10 of the earlier paper (Paintal, 1955b) well illustrates these arguments: in Fig. 10A, the first three cardiac cycles show that there are three impulses per cycle in the fibre with the small spikes. The receptor of this fibre was not stimulated by veratridine, which is evident when compared with the intense stimulation in B of the fibre with the large spike. But it can be seen in B that the small spike fibre now has 6 impulses/cycle. Both fibres are from type B left atrial receptors. Since the receptor of the fibre with the small spike is unaffected by veratridine, it must be concluded that the number and peak frequency of its impulses has increased in B owing to increased atrial filling, consequent on altered haemodynamic changes caused by slowing of the heart.

In B the lowest frequency of discharge in the large spike fibre is 88 impulses/sec and the peak frequency is 106/sec, i.e. the natural stimulus (atrial filling) although increased above that in A has raised the frequency by only 18 impulses/sec. In A, before the effect of the alkaloid had set in, the increase in frequency produced by atrial filling was about 50/sec. These facts suggest that the left atrial type B receptor was stimulated and simultaneously desensitized by veratridine.

Unfortunately, in the present series of experiments also, the left atrial pressure was not recorded so that an index of altered atrial filling is not available. It should be noted that pressure is not the natural stimulus of atrial
type B receptors (Paintal, 1953a; Henry & Pearce, 1956) and so it is of limited value. Even in the presence of a pressure record definite conclusions could not have been possible because changes in pressure and volume may not go hand in hand. There is at present no reliable method of recording quantitatively the changes in atrial volume which is the natural stimulus of the type B receptors.

Germitrine, about 25 µg, stimulated two left atrial type A receptors and one left atrial type B receptor. One left atrial type B receptor was unaffected by 45 µg of neogermitrine and 22 µg veratridine. 35 µg germerine stimulated one left atrial type A receptor. The responses of these receptors resembled those following veriloid, i.e. the injection–response time and duration of the discharge were of the same order as those after veriloid; also the peak-frequency/lowest-frequency ratio was markedly lowered after these alkaloids.

Two right atrial type B receptors were clearly stimulated by large doses of the alkaloids, i.e. 175 µg germitrine; the injection response times were 5.7 and 9.3 sec respectively and the duration was 1 min and 9 sec respectively. One of the receptors was also stimulated by 220 µg veratridine and the other by 350 µg neogermitrine. After 350 µg germitrine one of these two receptors was paralysed. A third right atrial type B receptor was also totally depressed after 315 µg germitrine. The total depression therefore resembles the depression observed in the pulmonary stretch receptors after these alkaloids.

The above observations show that the right atrial receptors can also be stimulated by veratrum alkaloids, but the quantities required to do this are much larger than those effective in stimulating the left atrial receptors. However, as concluded earlier (Paintal, 1955b), the right atrial receptors are not stimulated by small doses, so that they play no part in the Bezold reflex which is roused by much smaller doses of the alkaloids.

Occasionally one comes across an atrial fibre in which the activity is increased atypically by artificial inflation of the lungs (Paintal, 1955b). An example of such a fibre from a right atrial type B receptor is illustrated in Fig. 7 which shows the activity in it before and after germitrine. After opening the chest, the activity in this fibre consisted of bursts of impulses which were aroused by artificial inflation of the lungs; these bursts ceased soon after the inflation, to start again during the following inflation. An explanation for this behaviour can only be guessed at in the absence of a pressure record or a record of atrial volume. However, it may be assumed that atrial filling was the primary stimulus (subthreshold in this case) and that inflation of the lungs acted as a secondary stimulus to the receptor either by summatting with the subthreshold primary stimulus, or by increasing atrial filling. With this fibre on the recording electrodes, 175 µg germitrine was injected into the right atrium and the lungs were kept collapsed till 8.8 sec after the injection, when they were inflated (Fig. 7 B); this inflation precipitated the intense stimulation of the receptor as revealed by records B and C of Fig. 7. This response indicates clearly that the
stimulation of the receptor by germitrine was dependent on the initiation of activity in the receptor. This relation between activity and stimulation by the alkaloids has also been noted in pulmonary stretch receptors, and it is important to bear it in mind when considering the mechanism of stimulation by the alkaloids of veratrum.

Fig. 7. Stimulation of a right atrial type B fibre by germitrine in a cat with open chest. A shows the activity before injection of germitrine; note that activity in the fibre appears only during inflation of the lungs and ceases soon after. Record B begins 8·2 sec after injection of 175μg germitrine into the right atrium; it shows clearly that inflation of the lungs at 8·8 sec after injection of germitrine precipitated the stimulation by germitrine: C is a continuation of B. From above downwards in each record: e.c.g.; impulses in a right atrial type B fibre; time, 1/5 sec; and in A and B record of intratracheal pressure (retouched).
Gastric stretch receptors

The veratrum alkaloids stimulated thirteen out of fourteen gastric stretch receptors in the same doses as were required to stimulate the left atrial and ventricular pressure receptors, i.e. veriloid in amounts of 175–220 μg, veratridine 22 μg and germitrine 26 μg. The latency between injection and the beginning of stimulation tended to be shorter after veratridine, i.e. less than 10 sec; after both veriloid and germitrine it was usually between 10 sec and 20 sec. In two cases the latency after veriloid was about 1 min. The duration of stimulation was usually several minutes. In two fibres the duration of stimulation was 3.8 and 27 sec after 200 and 22 μg veratridine respectively. These observations are similar to those on the cardiac receptors.

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<th>Change produced by distension</th>
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* Negative sign indicates distension before injection of germitrine.

Desensitization of the receptors to distension of the stomach was seen after veriloid, veratridine and germitrine in 8 fibres on which this aspect of the problem was studied. Once again a preliminary stage of sensitization was not encountered. The progress of influence on the receptors is well illustrated in Table 1. 26 μg germitrine was injected at zero time and the effect of distension of the stomach with 200 ml. air from a respiratory pump before and after injection was recorded continuously. The resting discharge before stimulation by germitrine was zero and the frequency during the height of distension was 17–19 impulses/sec. Stimulation of the receptor set in at about 16 sec after the injection. Before the start of the next distension at 18'1 sec, the resting discharge was 9-6/sec but the peak frequency rose only to 20-4/sec, a difference of 10-8 impulses/sec, thus showing clearly that the receptor was desensitized from the beginning of stimulation. Thereafter, the increase in frequency produced by distension of the stomach became less and less (see last columns of Table 1) until the distension beginning at 40-3 sec after
injection of germitrine actually reduced the frequency of impulses; so did the next distension and the others following it. The response illustrated in Table 1 is typical of most responses of gastric stretch receptors to veratrum alkaloids. In this receptor missing of impulses, producing an irregular pattern of discharge, was also observed.

Cyclical activity resembling in some ways the cyclical activity seen in pulmonary stretch receptors was also seen in gastric stretch receptors. Fig. 8 illustrates this in one fibre after injection of 175 μg veriloid into the aorta; the stomach was not distended. It was noted that during the silent periods of the cycle the receptors were often totally unresponsive. The activity bore no relation to the intragastric pressure. Eventually, in some cases, the receptors became completely unresponsive and no impulses could be aroused in them by any means, in this resembling the pulmonary stretch receptors. Cyclical activity has been seen in gastric stretch receptors after certain chemical substances, e.g. adrenaline (Fig. 3 in Paintal, 1954a), but in these cases the peak frequency attained was low and the receptors responded normally during the silent phases of cyclical activity.

It is noteworthy that the peak frequency of discharge after the alkaloids never exceeded 70 impulses/sec; usually it was much less. This is in keeping with the responses of the receptors to distension of the stomach, in which the maximum frequency approaches 60 impulses/sec (Paintal, 1954a; Iggo, 1955).

Asphyxia. Once stimulation by the veratrium alkaloids had set in, asphyxia had no influence on the further course of events.

**Effect of calcium chloride and sodium citrate**

The effect of intra-atrial injections of 3% calcium chloride, on the stimulation produced by germitrine and germerine, was examined in the case of seven pulmonary stretch receptors. In one case 2.5 ml. was injected and this produced no change in the discharge precipitated by germitrine. This was probably owing to the quantity being too small, because in another fibre, where a similar dose had no effect either, increasing the amount to 5 ml. had the
typical effect described below. In all subsequent experiments, therefore, unless specifically mentioned, about 5 ml. was injected.

About 4–20 sec after the beginning of injection of calcium chloride the frequency of the continuous discharge aroused by germitrine began to decrease (Fig. 9) and within a few seconds the discharge ceased. Often, a few minutes after the injection of calcium chloride the continuous discharge tended

Fig. 9. Graph showing effect of injection of 5 ml. 3% calcium chloride on a pulmonary stretch receptor after it had been stimulated by 26 μg germitrine. Interrupted line indicates course of artificial ventilation. Graph shows clearly that the sensitivity of the receptor was increased after calcium chloride.

Fig. 10. Graph showing normal response of a pulmonary stretch receptor to inflation of the lungs with open chest, ●—●; response 54 sec after injection of 26 μg germitrine, ●—●●; response 1 min after injection of 5 ml. 3% calcium chloride, ○—○; and response 3–5 min after calcium chloride, ○⋯⋯○. Graphs show that calcium reduces to normal the discharge in the receptor by greatly reducing the continuous discharge of impulses and by increasing the sensitivity of the receptor. The graph of response 3–5 min after injection of calcium chloride shows that the continuous discharge has returned and the receptor is once again desensitized.
The most important action of calcium chloride was that, along with the cessation of the continuous discharge, the sensitivity of the receptors to inflation of the lungs was appreciably increased. In fact in some cases (e.g. Fig. 10) the sensitivity of the receptor returned to normal values. This figure also indicates that the threshold of stimulation by inflation of the lungs was also reduced a little after injection of calcium chloride. The influence of calcium lasted several minutes. In some pulmonary stretch fibres the responses were completely abolished after calcium was injected.

Calcium is known to stabilize excitable membranes, and its normal effect on receptors is to reduce their sensitivity. This was confirmed in one pulmonary stretch receptor in which calcium chloride reduced the peak frequency of discharge during 50 ml. inflation from 50 to 27 impulses/sec. In another fibre it prevented the marked sensitization of the receptor produced by trichlor-ethylene. It is, therefore, interesting that calcium should desensitize receptors normally and raise their sensitivity after germitrine.

Sodium citrate had an effect opposite to that of calcium. The effect was apparent after calcium had abolished the continuous discharge. Injection of sodium citrate at this stage precipitated the continuous discharge characteristic of veratrum alkaloids; 2.5–5 ml. of 2.5% sodium citrate was enough to produce this effect.

The effect of calcium chloride and sodium citrate on the atrial receptors was essentially the same as that on the pulmonary stretch receptors (Fig. 11). Within 5–9 sec after injection of calcium chloride into the right atrium the continuous discharge produced by germitrine or germerine was reduced, and a few seconds later the original type of rhythmic activity was restored. Thus the reduced peak-frequency/lowest-frequency ratio was increased. This response to calcium was seen in all three left atrial fibres on which the effect of this substance was tested. Injection of sodium citrate stimulated the receptor after the continuous discharge had been abolished by calcium chloride (Fig. 11 D). This also occurred after a latency similar to that following calcium chloride.

It is, therefore, clear that calcium chloride tends to reduce to normal the intense stimulation produced by the veratrum alkaloids, and sodium citrate to precipitate the discharge typical of the alkaloids after it has been reduced to normal by calcium chloride.

The activity in one left atrial type B fibre was greatly enhanced after injection of 2 ml. 1.2% KCl. No definite conclusions from this can be drawn as there was no way of gauging the direction of changes of atrial volume. However, the above observation is similar to those of Amann & Schaefer (1943) on cardiac receptors and of Jarisch et al. (1952) on carotid baroreceptors.
Injection of 2 ml. 1-2% KCl into the abdominal aorta stimulated five out of eight gastric stretch receptors; the resulting responses resembled those following injection of phenyl diguanide (see Paintal, 1954b) except that the intensity of stimulation was less.

Fig. 11. Impulses in a left atrial type B receptor. A is a normal record before injection of germitrine; B shows the intense stimulation by 90μg germitrine; the signal in this record marks the injection of 2 ml. 3% CaCl₂, which shows its influence in C (which is a continuation of B) by the unmistakable abolition of the continuous discharge produced by germitrine. Record D begins 3.5 sec after injection of 2 ml. 2.5% sodium citrate, which aroused the receptor after it had been acted on by calcium chloride as shown in C. From above downwards in each record: e.g.; impulses in fibres; time, 1/10 sec; in A intrapleural pressure; and in B injection signal.
Effect of trichlorethylene on pulmonary stretch receptors

The effects of volatile anaesthetics on the responses of pulmonary stretch receptors have been described in detail by Whitteridge & Büllbring (1944). One of the striking facts emerging from their investigation was that the volatile anaesthetics do not stimulate the pulmonary stretch receptors in spite of the considerable sensitization produced by them. Since this type of influence was in contrast to the effect of veratrum alkaloids on these receptors, a few experiments were done with trichlorethylene to confirm the observations of Whitteridge & Büllbring (1944). Some experiments carried out on the same receptors with both trichlorethylene and germitrine have provided valuable information about their contrasting influences on these receptors (Fig. 12).

The effects of trichlorethylene were examined on six pulmonary stretch receptors. In five of them the behaviour following this anaesthetic was identical with that described by Whitteridge & Büllbring (1944); one receptor was unaffected. Briefly, short exposures to the anaesthetic greatly sensitized the receptors, the peak frequency reached during peak inflation being often more than double that occurring before exposure to trichlorethylene. Longer
exposures totally abolished the responses of the receptors to inflation of the lungs. Activity in the fibres returned gradually after paralysis. This was accompanied by increasing degrees of sensitization until peak sensitization was reached; after this the sensitivity of the receptor returned to normal within a few minutes. This behaviour was unchanged after administration of atropine. At no stage, however, were the receptors stimulated by trichlorethylene, i.e. there was no activity of the receptors while the lungs remained collapsed.

DISCUSSION

The main points emerging from this investigation are as follows. (1) Veratrum alkaloids such as veratridine, germitrine, germerine and neogermitrine greatly stimulate and simultaneously desensitize pulmonary stretch receptors, atrial receptors and gastric stretch receptors. From suggestive evidence (Paintal, 1955b) it is concluded that the ventricular pressure receptors are similarly affected. The data of Jarisch et al. (1952) and Witzleb (1952) suggest that this is also true in the case of carotid baroreceptors. Indeed fig. 3 of Witzleb is highly suggestive. Fig. 3 of Dawes et al. (1951a) suggests strongly that veratrum stimulated and desensitized the pulmonary stretch receptors. (2) Calcium tends to reduce to normal the discharge following these alkaloids, and sodium citrate tends to precipitate the activity typical of these alkaloids after it has been reduced to normal by calcium chloride. (3) Trichlorethylene greatly sensitizes the receptors without stimulating them at all. Recently, Robertson et al. (1956) have shown that the carotid and aortic baroreceptors are similarly affected by volatile anaesthetics.

Other points which must be kept in mind in attempting to explain the mechanism of action by veratrum alkaloids are that the threshold of the physiological stimulus is not altered; that after the continuous discharge has set in irregularities due to missing of impulses often appear; that there are cycles of activity interspersed by periods of silence during which the receptors may be totally unresponsive or they may respond by a very rapidly adapting discharge; that the frequency at which the discharge sets in after a silence is often at its peak frequency (Fig. 6); that the persistent discharge may be terminated by its natural stimulus; and that anoxia or interference with the blood supply has no noticeable influence on the course of events following the injection of veratrum alkaloids.

Since veratrum is known to stimulate peripheral nerve fibres (see review by Krayar & Acheson, 1946) it is important first to establish the locus of action of the alkaloids. The behaviour of the pulmonary stretch receptors and the gastric stretch receptors strongly suggests that the veratrum alkaloids act on the ending itself. The most convincing observation in this connexion is the smooth increase in frequency of discharge that accompanies inflation of the lungs (Fig. 13), or distension of the stomach, during stimulation by the vera-
trum alkaloids. This smooth increase in frequency would not be expected to occur if the alkaloids acted on the nerve central to the ending, because the ending itself would then be bombarded antidromically and would be inactivated for a short while after each impulse (Matthews, 1933; Katz, 1950a). Further, if it is assumed that the ending was unaffected, the impulses arising from it would arrive at the region of the nerve stimulated by the alkaloids during various phases of its excitability cycle, some arriving there during its absolute refractory period; this would result in an irregular discharge of impulses.

![Fig. 13. Impulses in a pulmonary stretch fibre 2 min after injection of 26 μg germitrine. A shows that the increase in the frequency of discharge accompanying inflation with 100 ml. air occurs smoothly. B begins 3 sec after end of A: here also the change in frequency accompanying collapse of the lungs occurs smoothly. From above downwards in both records: impulse in a pulmonary stretch fibre; time, 1/5 sec; and intratracheal pressure. This figure provides evidence of action of the alkaloids on the ending itself.](image)

Action on the nerve itself and not on the ending will also not explain the marked unresponsiveness during the periods of silence of the cyclical activity. During this phase, when there are no impulses at all, it would be presumed that the excitatory effect of the alkaloids had died away and so a physiological stimulus would be expected to evoke a normal response; but it does not, and in fact there may be no response at all. A strong depolarizing block in the nerve could explain this behaviour, but if this were the case the high frequency rapidly adapting discharge produced by the physiological stimulus (Fig. 5) would not be allowed to pass through either. There is, therefore, little doubt that the alkaloids act on endings themselves and not on any portion of the nerve central to it.

The pulmonary stretch receptors (Adrian, 1933), the gastric stretch receptors (Paintal, 1954a) and the atrial type B receptors (Paintal, 1953a; Henry & Pearce, 1956) are all slowly adapting sensory receptors like the muscle spindle (Matthews, 1933). Like the muscle spindle, their natural stimulus is stretch
and so it is possible that the actual mechanism involved in the initiation of impulses in the muscle spindle would be the same in these receptors. This is assumed in the discussion that follows, which is based largely on the valuable observations of Katz (1950a, b), on the initiation of impulses in the frog's muscle spindle. Katz (1950a) found that a relatively prolonged negative after-potential follows the spindle spike potential, that stretching the muscle results in a local depolarization which is a function of the magnitude and the rate of the applied stretch, that local anaesthetics such as procaine and gross alterations of the ionic content of the fluid bathing the muscle had little, if any, effect on the local depolarization, although the initiation of impulses was completely depressed, and that the frequency of impulses is a linear function of the local depolarization.

It is not possible to say precisely what the veratrum alkaloids do to the receptors: but two likely mechanisms can be considered. First, it is possible that they depolarize the receptors, e.g. by altering the permeability of the membrane (Katz, 1950b; Gray & Sato, 1953) and thus enhance the local receptor potential of Katz (1950b) and Gray & Sato (1953). This potential has been labelled as the generator potential by Granit (1955). The onset of the continuous discharge in some receptors following the injection of the alkaloids is thus explained, and if it is assumed that this potential increases gradually the increase in the frequency of discharge can also be explained. Cathodic polarization is known to increase the discharge from muscle spindles (Edwards, 1955) and from labyrinthine endings (Lowenstein, 1955). The missing of impulses can be accounted for as being due to excessive depolarization (see Katz, 1950b). This depressing influence of strong cathodal depolarization has also been pointed out by Hodgkin (1948) in nerve, and recently Granit & Phillips (1956) have found it to occur in Purkinje cells during strong depolarization. It is possible that if the depolarization potential exceeds a critical level total block would occur and this could perhaps account for the silent phase of cyclical activity. However, there are other possible ways by which the silent phase associated with loss of responsiveness of the receptors to their natural stimulus could be produced.

To account for the desensitization, additional presumptions would have to be made, e.g. that the ratio of discharge frequency to the depolarization potential (see Katz, 1950b) is reduced after injection of the alkaloids, or that the amount of local depolarization produced by a standard physiological stimulus is reduced.

To account for the return towards normal of the responses of the receptors after calcium one would have to presume that the depolarization potential, which is unaffected normally by gross alterations in the ionic environment of the receptors (Katz, 1950b), is affected after the receptors have been influenced by the veratrum alkaloids. A similar reasoning is applicable to the effects of sodium citrate.
A direct depolarization of the receptors will not explain the type of response illustrated in Fig. 7 B because in this case the stimulation was clearly dependent on the occurrence of impulse activity in the receptor. If germitrine had depolarized the receptor directly in this and other similar cases, stimulation would have occurred without prior excitation by physiological stimuli.

The second possibility is that the veratrum alkaloids greatly enhance the negative after-potential of the receptors. This explanation is particularly attractive in view of the large body of evidence to prove that veratrum decidedly increases the negative after-potential in nerve fibres (Krayer & Acheson, 1948). Indeed, Acheson & Rosenblueth (1941) found that the amplitude of the negative after-potential was directly related to the dose of veratrine. Also that with increasingly severe veratrinization the repetitive response may eventually decline while the negative after-potential continues to grow more intense. Such a behaviour in the receptors is what one would expect from the results of the present investigation. This explanation will account for the type of response illustrated in Fig. 7 because the negative after-potential itself depends on impulse activity.

It must be emphasized that what has been discussed above is mere speculation, and it remains for future investigations to show whether the veratrum alkaloids enhance the local depolarization potential of the receptors, or the receptor negative after-potential.

Another phenomenon requiring explanation is the marked sensitization without any stimulation of the pulmonary stretch receptors and carotid baroreceptors (Robertson et al. 1956) by trichlorethylene and other volatile anaesthetics. The absence of a continuous discharge so characteristic of veratrum alkaloids can be explained by assuming that there is no persistent depolarization of the receptors. In this it would seem that the anaesthetics enhance the recovery processes associated with the initiation of impulses, i.e. the repetition frequency for a given depolarization produced by a standard physiological stimulus is increased. On the other hand, a less likely possibility is that the amount of local depolarization produced by the physiological stimulus is increased, leaving unaffected the relationship of the depolarization potential to the impulse frequency described by Katz (1950b). Much more information is needed before any definite conclusion can emerge, but it is interesting that the observations of Katz provide a theoretical basis for these two ways of sensitizing a receptor. The above and certain other aspects of excitation of mechanoreceptors by chemical substances, e.g. the role of potassium in the excitation of sensory receptors by veratrum alkaloids, have been discussed elsewhere (Paintal, 1956).
SUMMARY

1. The influence of certain veratrum alkaloids on atrial and pulmonary and gastric stretch receptors has been studied by recording the action potentials in single vagal afferent fibres arising from these receptors. Of the four alkaloids, germitrine, germerine, neogermitrine and veratridine, germitrine was most frequently used and the description given is typical of the actions of germitrine.

2. The veratrum alkaloids greatly stimulated and simultaneously desensitized the receptors to their natural stimuli. The right atrial receptors were stimulated only by large amounts of the alkaloids.

3. The alkaloids produced cyclical activity in pulmonary and gastric stretch receptors. During the active phase the continuous discharge could be terminated by the natural stimulus of the receptors. In many fibres irregular discharges appeared owing to dropping out of impulses. During the inactive phase the responses of the receptors were either greatly depressed or abolished. Injection of large amounts of the alkaloids paralysed the receptors completely.

4. The threshold and adaptation rate of the receptors were either unchanged or increased.

5. Calcium chloride greatly reduced or abolished the continuous discharge produced by the alkaloids in atrial and pulmonary stretch receptors; in the latter the sensitivity of the receptors was simultaneously increased. Sodium citrate precipitated the continuous discharge after it had been abolished by calcium chloride. Potassium chloride stimulated some gastric stretch receptors and it seemed to have sensitized atrial receptors.

6. The observation of Whitteridge & Büllbring (1944) that trichlorethylene greatly sensitizes the pulmonary stretch receptors was confirmed. This sensitization was not accompanied by stimulation of the receptors.

7. The evidence indicates that the alkaloids act on the endings themselves. The possible mechanisms responsible for the contrasting effects of veratrum alkaloids and trichlorethylene, i.e. stimulation and desensitization by the alkaloids, and sensitization without stimulation by trichlorethylene, have been discussed.

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