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SOME ACTIONS OF SUBSTANCE P ON THE CEREBRAL CORTEX AND THE BRAIN STEM RETICULAR FORMATION

Since the basic investigation on the properties of SP by von Euler and Gaddum (1931) numerous experiments have provided evidence that the polypeptide, besides its well-known action on smooth muscle organs, exerts a definite influence on several central nervous functions. Among the cerebral effects hitherto described a general decrease of vigilance following a parenteral application of the substance represents a most striking phenomenon which has been demonstrated in various animal species [cf. v. Euler and Pernow (1954, 1956), Zetler (1956, 1959), Stern (1959), Stern and Huković (1958), Stern and Dobrić (1957) et al.]. In spite of the fact that this action of SP implies important problems both from a neurophysiological and a pharmacological point of view, little is known as yet about its fundamental mechanisms. The following experiments were designed to establish whether changes of cortical and reticular activation processes are involved in generating the sedative effect. The results obtained provide a first and, surely, limited insight into the origin of the quieting phenomenon which, as a whole, is probably a very complicated one. With respect to previous findings concerning the correlation between behavioural activity changes of the animal and cortical d. c. deviations (Caspers, 1959, 1961; Caspers and Schulze, 1959) the steady potential of the cerebral cortex (d. c. component) served as the principal indicator of the investigated SP actions. A more detailed account of the general experimental procedures adopted will be given in the following sections of this paper.

Methods

The investigations were performed on non-anaesthetized, freely moving rats with chronically implanted electrodes. The steady potential of the cerebral cortex was led from various motor, sensory and association areas of the intact surface and recorded against a common reference point in the front portion of the muzzle which is sufficiently indifferent. In each of the subsequent figures an upward deflection of the d. c. records indicates a negativity of the active electrode on the cortical surface, and vice versa. Further details of the d. c. recording techniques adopted have already been published elsewhere [Caspers (1959, 1961); Caspers and Schulze (1959); Caspers and Stern, (1961)]. Apart from the d. c. potential of the cerebral cortex the motor activity of the animal, the conventional EEG and the unit discharges in various parts of the midbrain reticular formation were recorded. In most of the experiments additional records were taken from extrapyramidal motor structures of the brain stem, particularly from the nucleus ruber and its surrounding areas.

Throughout the experiments 3 SP-preparations containing 6.7; 16.5 and 20 units SP per mg were available. As a rule, the (diluted) substance was administered intraperitoneally (i. p.) in amounts ranging from 500 to 10,000 U./kg body weight. According to the results of control experiments the typical effects of SP described in the following sections of this paper are either extinguished or at least greatly reduced by a preceding incubation of the substance with trypsin. This finding indicates that the main effective component of the applied inhomogenous preparations actually represents a polypeptide.

Results

I. **Normal variations of the cortical d. c. potential in the freely moving rat.** — In the freely moving rat the cortical d. c. potential shows considerable fluctuations which are synchronized with behavioural activity changes of the animal. During the transition from wakefulness to sleep, for instance, the base line of the d. c. record shifts to the positive side relative to the mean waking level. An arousal reaction, on the other hand, is invariably associated with a negative displacement of the surface steady potential. In the waking animal similar negative d. c. deflections occur with spontaneous (orienting) movements or can be induced by various sensory stimuli. The extent, voltage and time course of such peripherally evoked d. c. shifts depend on the intensity, on the repetition rate and on the modality of the stimulus applied. Negative deflections of considerable amplitude are particularly released by tactile stimulations of the whiskers and of frontal head areas. In experiments on less tamed rats similar high voltage d. c. deviations to the negative side which correspond to the behavioural attention reaction can be initiated by approaching the animal to a critical distance. As compared with tactile stimuli tone pulses, for instance, produce rather small and often circumscribed d. c. reactions which rapidly adapt. Amplitude and extent of such acoustically evoked shifts, however, tend to increase if conditioned stimuli are employed. According to our present experience such conditioned auditory d. c. reactions are, in addition, less susceptible to adaptation.

Some of the d. c. findings summarized above are illustrated in Fig. 1 which represents an original record of cortical d. c. and a. c. potentials at a low paper speed in an awake and freely moving rat. In general agreement with the results of previous investigations which have been described in detail elsewhere (Caspers et al.) the tracings indicate that the d. c. component reflects both the spontaneous and the evoked activity changes of the cerebral cortex more precisely than the conventional EEG. In close accordance with the behavioural reactions of the animal voltage, extent and duration of an induced d. c. shift are obviously related to the amount of »information« which a given stimulus contains. As could be concluded from earlier experiments (Caspers and Schulze, in press), the widespread d. c. displacements are mediated, at least preferentially, by the brain stem reticular formation. For these reasons the steady potential of the cerebral cortex can be regarded as a suitable indicator for analyzing the actions of SP on the reticulo-cortical system.

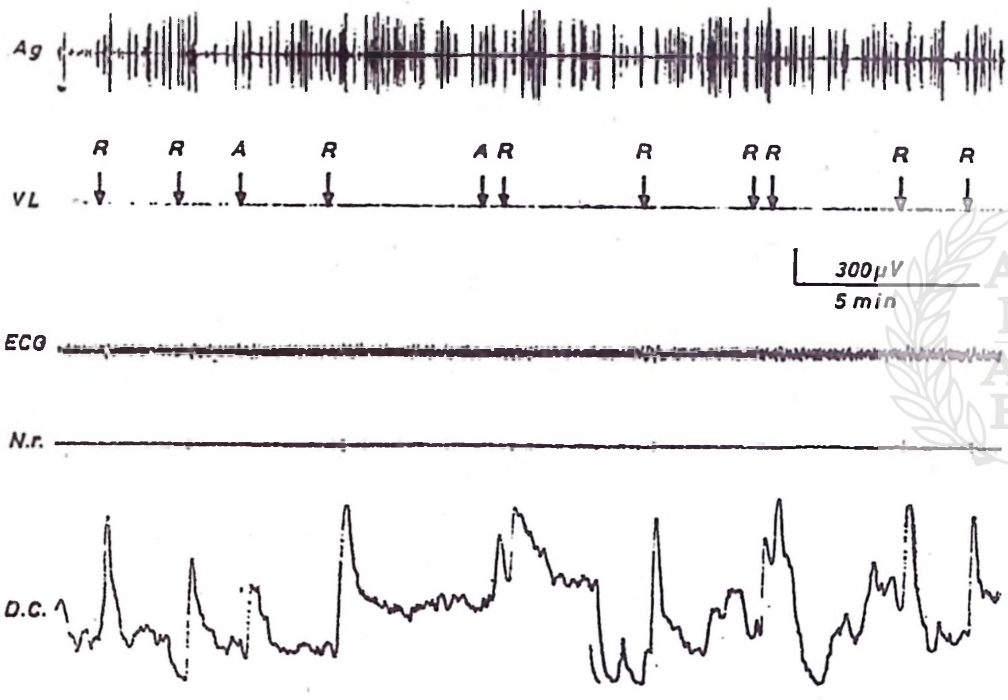

FIG. 1

Simultaneous recordings of the cortical d. c. potential (D. C.), the unit activity of the nucleus ruber (N. r.), the conventional electrocorticogram (ECG) and of the motor activity of the animal (Ag; tracing of a vibration box). The curve N. r. was additionally recorded by an oscilloscope. The moment of stimulation is indicated by arrows on the comparison line (VL). R: Tactile stimulation of the vibrissae. A: Approach by the experimenter. As in the subsequent figures an upward deflection of the d. c. curve indicates a negativity of the active electrode on the cortical surface. In this experiment a subsequent treatment of the animal with 3 mg chlorpromazine effected a complete suppression of the normal d. c. fluctuations thus proving their biological origin.

Some experimental results concerning the fundamental mechanisms possibly involved in generating the d. c. component have already been discussed in previous papers (see Caspers et al.). The available data support the preliminary hypothesis that the d. c. recordings represent a widespread integration of polarization changes in the mass of apical dendrites and/or other cortical elements which are initiated, for instance, by afferent neuronal impulses or by humoral agents.

II. **The action of SP on the cortical d. c. potential.** — After an i. p. application of 4,000—8,000 U./kg body weight the steady potential of the cerebral cortex shows a distinct p o s i t i v e shift which is usually associated with an increase in voltage and duration of the EEG waves. Apart from quantitative differences these bio-electrical effects, therefore, resemble both the d. c. and the EEG alterations occurring at the onset of natural sleep. The positive displacement of the d. c. component elicited by SP starts after a short latency and reaches a maximum value of 2—4 mV within 10—15 min. after the injection (Fig. 2). The d. c. shift having flattened and finally ceased, the base line remains deviated to the positive side for a variable time and then gradually returns to the initial level.

Monophasically positive d. c. reactions as described above represent a typical SP effect. They particularly occur in either case in which a relatively small amount of SP ranging from about 2,000 to 4,000 U./kg is administered. Higher doses of SP, however, often release multiphasic d. c. deviations. In these experiments the initial positive shift is followed by a rather strong negative d. c. deflection which compensates and sometimes even exceeds the preceding positivity. An example of such a finding is illustrated in Fig. 2. The basic mechanisms of the secondary negative shifts which are due to extracerebral actions of SP will be discussed in a later section of this paper.

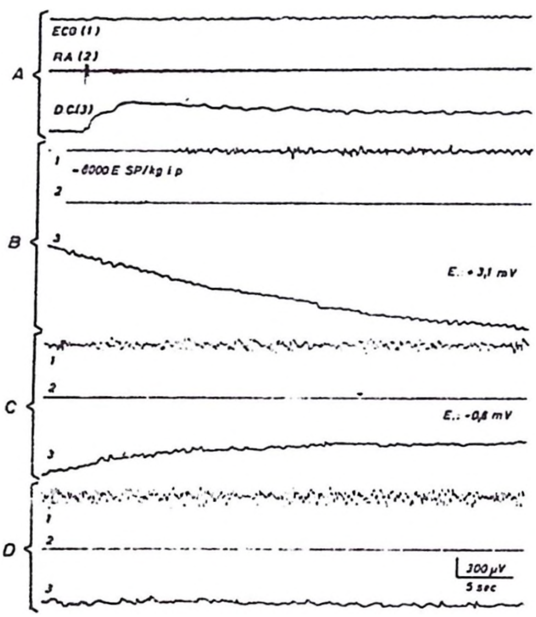

FIG. 2

The action of 8,000 U./kg SP i. p. on the electrocorticogram (ECG; 1), on the activity level of midbrain reticular neurons (R. A.: 2) and on the base line of the cortical steady potential (D. C.; 3). Tracing 2 was simultaneously recorded by an oscilloscope. A: Normal record with a spontaneous activation of the animal at the beginning of the curve. B: Positive shift of the steady potential immediately after the application of SP. C: Secondary negative d. c. shift 4 min. later. The letter E in B and C indicates the final amount of the positive and negative d. c. deviation. D: Stable base line of the d. c. component about 18 min. after the SP application.

The base line deviations of the steady potential which were hitherto discussed are always associated with an extinction or a strong reduction of the spontaneous d. c. fluctuations occurring before the application of SP. This stabilization of the d. c. component corresponds to the behavioural sedative effects elicited by the polypeptide. In addition to the spontaneous shifts the evoked negative d. c. deflections, too, are either abolished or decreased. Fig. 3 demonstrates a temporary extinction of a peripherally induced negative d. c. displacement after the i. p. application of 8,000 U./kg body weight. The various tracings in Fig. 3 furthermore indicate that the suppression of the evoked negative d. c. shifts is closely related to another SP effect. In the freely moving animal an induced rise of the cortical excitation level reflected in an extensive and high voltage d. c. deviation is frequently associated with an activation of the extrapyramidal motor system of the brain (Fig. 3 A 1). This concomitant activation of motor structures is reduced by SP and usually disappears when the evoked d. c. shift reaches a critical minimum (see also Fig. 4). This finding points to a certain causal link between the basic mechanisms responsible for the cortical d. c. displacements as well as for the activation of the extrapyramidal system. According to the results of previous experiments the brain stem reticular formation possibly plays an essential rôle in mediating both these effects (cf. p. 31). In the majority of experiments the SP actions which were described above last about 1 hour. Both the duration and the intensity of the d. c. and EEG changes are, however, subject to some variation which is not due to differences in the applied dose.

In accordance with the experiment illustrated in Fig. 3 an i. p. application of 8,000 U./kg nearly always leads to a rapid and temporarily complete depression of the various evoked d. c. shifts. In order to study the process of extinction in greater detail fractionated SP injections up to the same final amount were therefore employed. According to these experiments the first visible effect usually obtained with 1,000—2,000 U./kg i. p. consists in a moderate slowing of the evoked potential rise. Increasing doses of SP additionally reduce the extent, the voltage and the duration of the d. c. deflections. Finally, the small persistent shifts of the cortical steady potential use to be restricted, more or less, to the primary projection field pertaining to the stimulus applied. Fig. 4 demonstrates a partial reduction of a peripherally induced negative d. c. displacement after i. p. application of 4,000 U./kg.

The employment of fractionated SP-injections furthermore reveals remarkable differences concerning the susceptibility of the negative d. c. deflections elicited by various peripheral stimuli. If the reduction of each negative shift is measured in per cent of its mean normal value an acoustically evoked d. c. reaction, for instance, proves by far more susceptible to SP than the negative deflections induced by tactile stimulations of the animal. Among the various d. c. deviations elicited by natural stimuli conditioned reactions of the steady potential are particularly resistent to SP. This statement is illustrated in Fig. 5. In these records the proper stimulation response of the d. c. component (R) is preceded by a definite negative shift elicited by approaching the animal (A). In this special experiment the initial d. c. deviation represents the bioelectrical sign of a conditioned flight response established by the method of punishment. According to the tracings summarized in Fig. 5 the primary alarming reaction of the d. c. component (A) is clearly less reduced by SP than the unconditioned stimulation response (R). The concomitant activation of extrapyramidal motor structures, however, temporarily disappears even in this case (Fig. 5 B—C). As compared with the conditioned flight effects the spontaneous »approaching« reactions of the steady potential which frequently occur

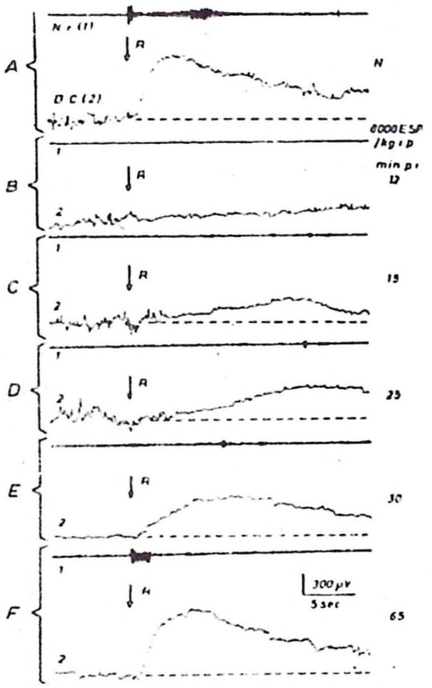

FIG. 3

The influence of SP on the neuronal activity of the nucleus ruber (N. r.; 1) and on the negative shift of the cortical steady potential (D. C.; 2) evoked by a tactile stimulation of the vibrissae (R). A: Normal stimulation responses. B-F: Extinction and recovery of the natural stimulation reactions after an i. p. application of 8,000 U./kg body weight.

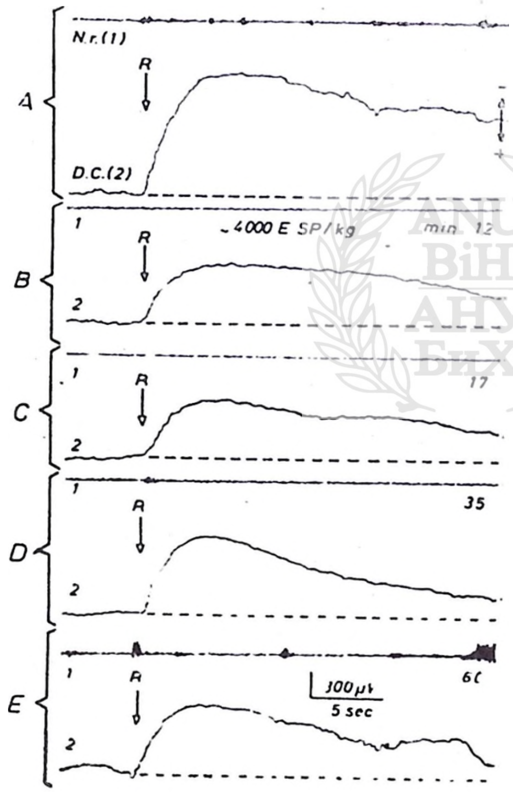

FIG. 4

The action of SP on the neuronal activity of the nucleus ruber (N. r.; 1) and on the negative shifts of the cortical steady potential (D. C.; 2) evoked by a tactile stimulation of the vibrissae (R). A: Normal stimulation responses. B-E: Reduction and recovery of the normal stimulation reactions after an i. p. application of 4,000 U./kg body weight.

also in normal animals without a special training (see Fig. 1) are somewhat less resistent to SP. Their reduction runs approximately parallel to the depression of the negative shifts evoked by tactile stimulations of the animal. As a whole, the alterations of various evoked d. c. shifts elicited by SP correspond in several aspects to the modification of conditioned and unconditioned behavioural phenomena which has been reported by Stern (1959).

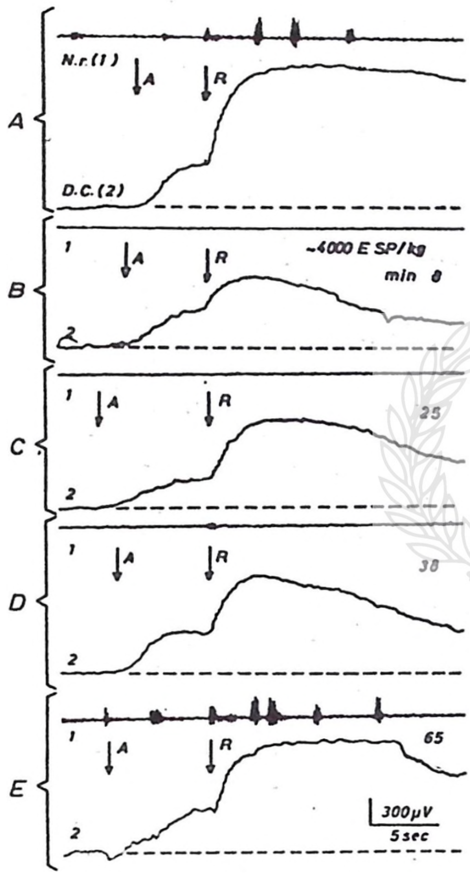

FIG. 5

The influence of SP on the neuronal activity of the nucleus ruber (N. r.: 1) and on the negative shifts of the cortical steady potential (D. C.; 2) evoked by approaching the animal (A) and by a subsequent stimulation of the whiskers (R). A: Normal stimulation responses. B-E: Reduction and recovery of the stimulation reactions after an i. p. application of 4,000 U./kg body weight. (for further explanations see text!)

III. **Some actions of SP on the behaviour of the rat.** — The changes of the cortical steady potential released by different doses of SP are closely related to typical behavioural phenomena which have already been observed in other animal species [v. Euler and Pernow (1956), Zetler (1956, 1959), Stern and Dobrić (1957), Stern and Huković (1958), Stern (1959) et al.]. The special features of the sedative effect depend on the applied dose. Smaller amounts of SP (i. e. 1,000—2,000 U./kg i. p.) usually cause a distinct reduction of spontaneity which can be distinguished from a physiological period of inactivity by a preceding analyses of the normal activity cycle of the individual. In contrast to the spontaneous movements the motor responses of the animal to peripheral stimulations are, at first, scarcely altered. This statement especially proves true if »meaningful« stimuli are employed (cf. p. 35). Higher doses of SP (i. e. about 4,000 U./kg) usually effect a complete depression of any spontaneous motor activity, the reaction of the animal to peripheral stimuli concurrently being reduced. Such (medium) doses of SP occasionally provoke certain automatisms consisting, for instance, in chewing movements or in periodical risings of the head. Large amounts of SP exceeding 8,000—10,000 U./kg body weight finally cause a stuporous state with a temporary extinction of all spontaneous and induced motor actions. These findings are in general agreement with the observations made by von Euler and Pernow (1956) in cats and rabbits. In the majority of experiments the strong sedative effects produced by high doses of SP last about 30—45 min. and then vanish within the course of the subsequent hour. During this period the normal fluctuations of the cortical steady potential gradually reappear.

IV. **The direct actions of SP on the cerebral cortex.** — According to the results of previous investigations the extensive shifts of the cortical steady potential occurring with spontaneous or induced activity changes of the animal are mediated preferentially by the brain stem reticular formation (cf. p. 31). Therefore, the reduction of the evoked negative d. c. deflections could be due, in principle, both to a direct influence of SP on the cortical generator structures and to an inhibition of reticular neurons. A further analysis of the fundamental processes involved in the depression of the d. c. shifts can be achieved by comparing the activity changes of the cortex produced by a local and by an i. p. administration of SP.

As already observed in preceding experiments direct applications of SP to the cortical surface, excluding secondary actions of the substance via subcortical and extracerebral structures, exert a strong influence on the bioelectrical activity of the cortex (cf. Caspers and Stern, 1961). Various amounts of SP ranging from 1 to about 10 units per 10 mm^2 cortical surface cause a monophasically positive shift of the steady potential. The positive d. c. deviation is accompanied by typical variations of the direct cortical response (DCR) to a single electrical stimulus (so-called dendritic potential). Employing currents of low intensity a local application of SP always effects a distinct reduction of the DCR. The same amount of the substance. on the other hand, causes a significant increase of the DCR if stronger, »supra-threshold« stimuli are applied. Opposite SP effects of exactly the same kind depending on the current intensity are expressed in the negative d. c. displacements induced by a direct activation of the cortical surface with series of electrical pulses. In contrast to these d. c. deviations the natural shifts released by physiological stimuli are constantly depressed at the site of application. On that account the neuronal afferent impulses impinging upon the cerebral cortex act like a weak direct electrical stimulation. In connection with the results of additional polarization experiments these findings, moreover, suggested that SP establishes a hyperpolarization of the structural elements which are responsible for generating both the d. c. component and the DCR.

Compared with the local actions of SP the various activity changes of the cortex occurring after an i. p. injection of the substance show some peculiarities. While a direct application of SP to the cortical surface, in either case, produces a monophasically positive deviation of the d. c. component intraperitoneal injections of the substance often release a secondary negative shift which sometimes even exceeds the initial positive displacement. An example of such an experiment was already illustrated in Fig. 2. Likewise, the changes of a supra-threshold direct cortical response following an i. p. application of SP may be entirely different. As a rule, medium amounts of the polypeptide up to 4,000 U./kg i. p. cause a distinct increase of the DCR (Fig. 6). This effect is identical with the local actions of the substance. Higher doses of SP, on the other hand, often reduce or abolish the DCR even if maximum stimuli are employed. An example of such a record is demonstrated in Fig. 7. The extinction of the (supra-threshold) DCR which was never observed after a local application of SP is closely related to the secondary negative shifts of the d. c. component and promptly abolished by an anodal polarization of the cortical surface. The negative deviations of the steady potential, on their side, always appear in connection with a lowering of blood pressure elicited by a sufficient amount of SP. They rather constantly start whenever the mean systemic pressure declines to a certain minimum value which varies to some extent from one individual to another. Negative d. c. shifts of exactly the same form which are accompanied by a reduction or a suppression of the DCR also occur when the critical fall in blood pressure is initiated by substances other than SP. Taking into account the results of earlier experiments, it may be assumed that the negative displacements of the steady potential depend upon an increasing depolarization of the cortical generator membranes released by hypoxia. On this condition the negative DCR which is induced by an additional electrical stimulus necessarily decreases or disappears independently of the stimulus strenght employed. The negative shift of the surface steady potential as well as the reduction of the DCR, therefore, obviously represent secondary effects of SP which are mediated by changes in circulation. Consistent with the results of previous experiments (cf. Caspers and Stern, 1961) both the positive d. c. deviations and the increase of the DCR, on the other hand, can be attributed preferentially to a direct action of SP on the cerebral cortex.

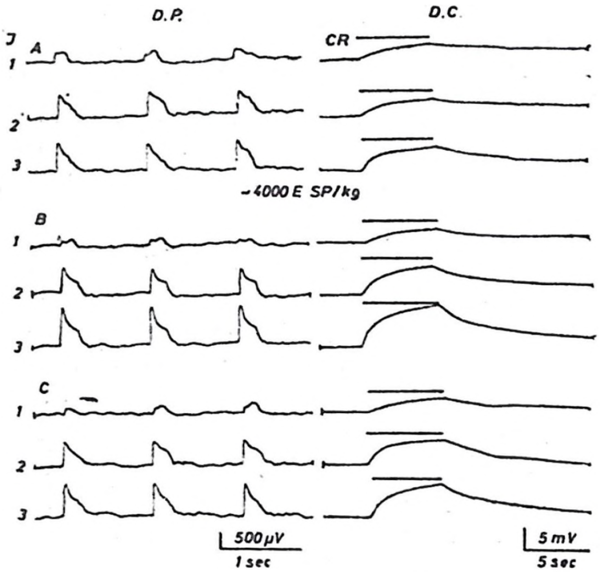

FIG. 6

The action of SP on the direct cortical response (so-called dendritic potential; D. P.) and on the negative shift of the steady potential (D. C.) induced by an electrical stimulation of the cortical surface with a 20/sec. impulse series. The time of stimulation is indicated by horizontal lines (CR) above each d. c. record.

A: Normal D. P. and D. C. responses to electrical stimuli of increasing intensity (I 1; I 2; I 3). B-C: Variations and recovery of the normal stimulation responses of the cerebral cortex 10 min. (B) and 50 min. (C) after an i. p. injection of 4,000 U./kg body weight. (for further explanations see text!)

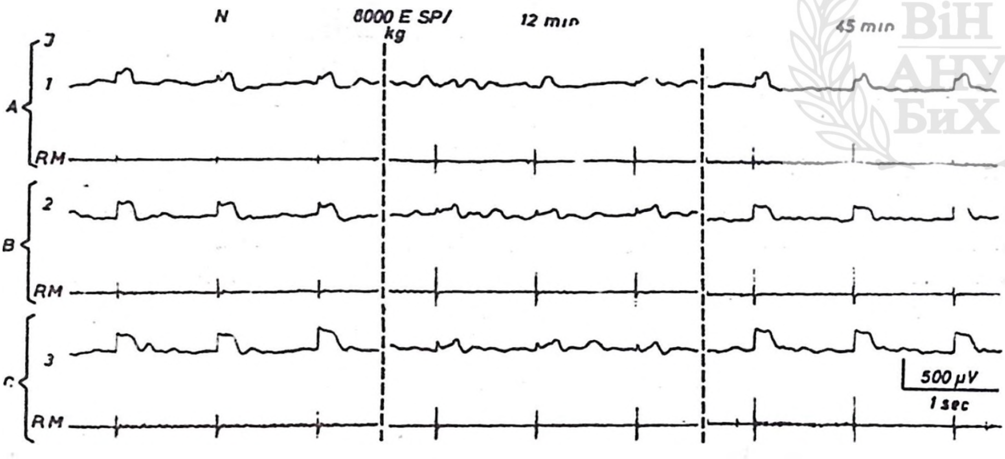

FIG. 7

The action of larger amounts of SP on the direct cortical response to a single electrical stimulus. The moment of each stimulation is indicated by the vertical lines in RM. N: Normal response of the cerebral cortex to stimuli of increasing intensity (A, B, C). The medium column of the figure demonstrates a considerable reduction of the normal response at all current intensities 12 min. after the i. p. application of 8,000 U./kg body weight. The right column of tracings shows a recovery of the effect 45 min. after the injection. (for further explanations see text!)

According to the results discussed above any reliable conclusion concerning the main site of the SP-actions can be derived only from those experiments in which a substantial lowering of blood pressure is missing or in which it is possible to compensate the effects on circulation by means of medicaments which do not influence, at least to a measurable extent, the functions of the brain themselves. These special conditions proved accomplished, more or less, in a total of seven tests. As could be calculated from the various records injections of SP just sufficient to abolish each evoked negative d. c. shift release a positive deviation of the d. c. base line up to a maximum of 4 mV. The concurrent increase of the direct cortical response to a suprathreshold stimulus amounts to 30 per cent of the initial value. A local administration of SP, on the other hand, which also suppresses all of the evoked negative d. c. deflections at the site of application causes a positive displacement of the d. c. base line up to 8 mV and increases the DCR up to 80 per cent of its mean normal voltage. These comparative measurements indicate that the reduction or extinction of the peripherally evoked d. c. deviations following an i. p. injection of SP cannot be completely explained by a mere cortical action of the substance. A significant part of these effects is apparently due to an inhibition of reticular activity.

V. **Some actions of SP on the activity of the brain stem reticular system.** — The assumption that SP is apt to inhibit reticular neurons was examined, at first, in some additional stimulation experiments. As already observed by various authors [cf. Arduini et al. (1957), Goldring and O'Leary (1957), Brookhart et al. (1958), Caspers (1959)] high frequency stimulations of the brain stem reticular system elicit quite similar negative shifts of the cortical steady potential as are released by natural stimuli. According to the results of more recent investigations two different types of the cortical d. c. response to an electrical activation of reticular structures are to be distinguished (Caspers and Schulze, in press). Besides steep deviations of the steady potential which arise from the base line immediately at the commencement of the reticular stimulation, flat shifts of considerable latency occur. An example of the slow type of stimulation response is illustrated in Fig. 8. This form of reaction is usually obtained with the electrodes located in the lateral portion of the mesencephalic reticular system, while the first, short latency type predominates in the median part. Both forms of the electrically evoked d. c. displacements prove rather susceptible to SP, the slow type being the most sensitive one. As a rule, it disappears after an i. p. application of 4,000—8,000 U./kg (Fig. 8). In either case, however, the negative shifts of the cortical steady potential induced by a reticular activation are completely depressed when a direct stimulation of a thalamic relais nucleus, for instance, still effects distinct d. c. alterations. These findings suggest that the reticular system is particularly sensitive to SP, and that a lowering of the reticular excitation level may actually be involved in the production of the sedative effects which are reflected in the d. c. alterations at the cerebral cortex. The records summarized in Fig. 9 yield a direct evidence of this interpretation. The subsequent tracings demonstrate that both the basic activity level and the stimulation response of reticular neurons are clearly depressed by SP. The normal responsiveness of the recorded units is gradually restored within the course of 1 hour following the application of SP. In this experiment the recovery curve of the reticular activation runs parallel to the recreation of the peripherally evoked d. c. shifts. As a whole,

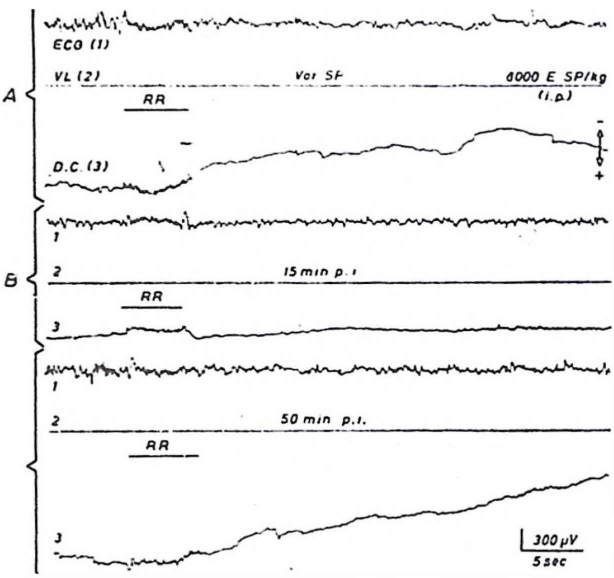

FIG. 8

The action of SP on the electrocorticogram (ECG) and on the negative shift of the cortical steady potential (D. C.: 3) released by a direct stimulation of the lateral portion of the midbrain reticular formation. VL (2) presents a comparison line. The period of reticular stimulation in each tracing is indicated by the horizontal lines RR. A: Normal stimulation response. B-C: Extinction and recovery of the evoked (long-latency) deviation of the cortical d. c. component after the i. p. application of 8,000 U./kg body weight SP.

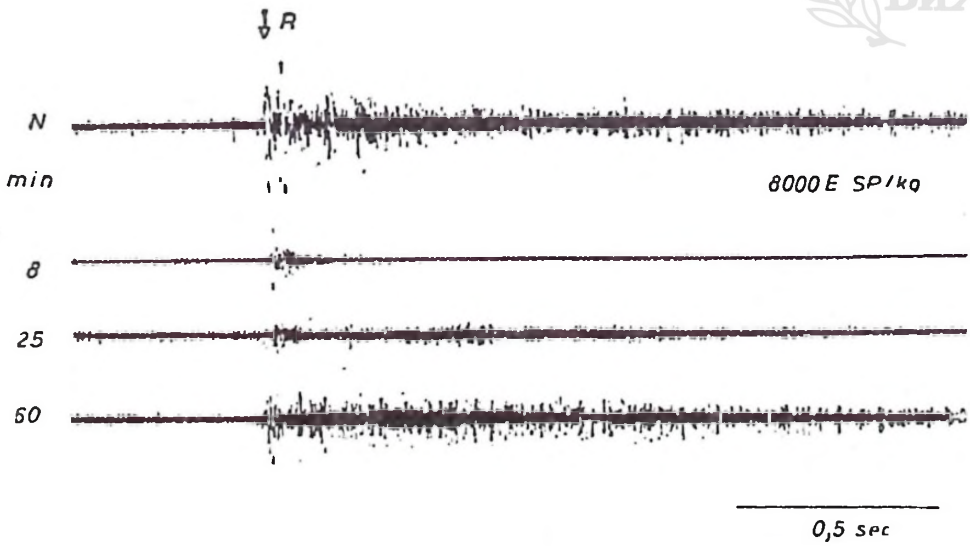

FIG. 9

The action of SP on the unit discharges of neurons in the midbrain reticular formation evoked by a tactile stimulation of the vibrissae. The moment of stimulation in each tracing is indicated by the arrow R. Both the level of the background activity and the normal stimulation response (N) of reticular neurons recorded with a 50 µ electrode are strongly reduced after the i. p. application of 8,000 U./kg. The normal response has recovered approximately after 60 min.

the investigations allow to conclude that the depression of cortical excitation processes following an i. p. injection of SP can be explained only in part by a direct action of the polypeptide on the cortical generator structures. A significant component of the sedative effects reflected in the extinction of the evoked cortical d. c. shifts consists of an inhibitory action of SP on reticular neurons.

Summing up, the experiments have shown that the depression of spontaneity released by a parenteral application of SP is actually accompanied by typical activity changes in the reticulo-cortical system. As could be concluded from the various d. c. alterations both the basic activity level and the responsiveness of the cerebral cortex to different natural stimuli are greatly reduced. With respect to the stimulation reaction of the reticulo-cortical system, SP acts, as a whole, in a way as if the amount of »information« implied in a given peripheral stimulus had been diminished. The fundamental neurophysiological mechanisms responsible for these SP effects are, as yet, unknown. Some experimental data support the hypothesis proposed, for instance, by Zetler (1959) that the polypeptide represents a transmitter substance at certain inhibitory synapses. The conclusion of Caspers and Stern (1961) that SP causes a hyperpolarization of neuronal structures would favour this interpretation. A direct evidence of the assumption that the polypeptide might be involved in the transmission of inhibitory impulses is, however, entirely lacking. At the present stage of our knowledge we would prefer to assume that SP represents a hyperpolarizing, permanently active agent, the neuronal actions of which being more diffusely distributed.

Summary

The question of how SP is influencing the stimulation processes in the cortex and in the reticular formation of the brain stem has been investigated on freely moving rats with chronically implanted electrodes. The direct potential component (Bestand-potential) of cortical bioelectric activity, the so called dendritic potential, the spontaneous waves of the EEG and the neuronal discharges in the mesencephalon reticular structures served as criteria for SP action. Bioelectric effects and the known sedative action of SP have been correlated simultaneously. The experiments have shown:

(1) After i. p. administration of about 2,000—4,000 U./kg SP the cortical direct potential is shifted to the positive side of the initial value for several mV. The assuming of more positive potentials by the cortex is connected, similarly as in natural sleep, with an increase of the so called dendritic potential and the spontaneous waves of the EEG. After administration of h i g h e r doses of SP the primary positive shift may quickly change over into a negative one. Such negative potential shifts in the cortex which are lacking when SP is applied locally indicate a close relationship to the blood pressure reduction

produced by higher doses of SP. They obviously represent secondary effects in the cortex, depending on extracerebral action of SP.

(2) The shift of Bestandpotential appearing after an injection of SP is always coupled with a reduction of fluctuations of negative direct potential which, in awake, freely moving animals, results from sensory stimulation. The depression of response to stimuli, measured in per cent of the initial value, increases with increasing doses of SP. The depression, further, depends on the kind of stimulus applied. With equal dosage of SP the per cent reduction of direct potential fluctuations is generally the smaller, the more »information« for the individual under examination there is contained in the sensory stimulus.

(3) The inhibition of cortical stimulation processes as reflected in direct potential tracings is partly due to direct action of SP in the cortex. But according to comparative measurements carried out with intraperitoneal and local administrations of SP this inhibition cannot entirely be explained by its direct action. Another essential cause of cortical inhibition effects rather lies in a lowering of the reticular stimulation level. As shown by direct recording of neuronal discharges in the brain stem reticular formation the spontaneous activity, as well as the response to stimulation of reticular structures are considerably reduced by SP.

From the results obtained it might be concluded that the suppression of stimulation processes in the reticulo-cortical system takes a significant part in the appearance of the well-known sedative effects of SP.

NEKI EFEKTI SP U MOŽDANOJ KORI I RETIKULARNOJ FORMACIJI MOŽDANOG DEBLA

Na slobodno pokretljivom štakoru kome su kronično implantirane elektrode istražen je način djelovanja SP na stimulativne procese u moždanoj kori i retikularnoj formaciji moždanog debla. Kao kriteriji za prosuđivanje djelovanja SP poslužili su: istosmjerna komponenta (Bestandpotential) bioelektričke aktivnosti moždane kore, zatim tzv. dendritni potencijal, spontani talasi EEG i neuronska izbijanja u retikularnoj formaciji. Istovremeno su ove bioelektričke manifestacije povezane s poznatim sedativnim djelovanjem SP na ponašanje životinja. Eksperimenti su pokazali:

(1) Poslije intraperitonealne aplikacije oko 2.000 do 4.000 jed./kg SP, istosmjerni kortikalni potencijal pomiče se za nekoliko mV na pozitivnu stranu ishodne vrijednosti. Pojava pozitivnog potencijala u moždanoj kori ide u korak s povećanjem tzv. dendritnog potencijala i spontanih talas EEG slično kao kod prirodnog spavanja. Poslije v e ć i h doza SP može prvobitni pozitivni pomak kortikalnog potencijala ubrzo preći u negativni. Nastup takvih negativnih potencijala moždane kore, kojih nema pri lokalnoj aplikaciji SP, usko je povezan sa smanjivanjem krvnog pritiska što ga izazivaju veće doze SP. To su, dakle, očito sekundarni efekti u moždanoj kori, uvjetovani ekstracerebralnim djelovanjem SP.

(2) Pomaci istosmjernog kortikalnog potencijala, koji nastupaju poslije injekcija SP, uvijek su skopčani sa smanjivanjem fluktuacija negativnog istosmjernog potencijala, koje su kod budne, slobodno pokretljive životinje posljedica senzornih podražaja. Smanjivanje reakcije na podražaj, izraženo u procentima početne vrijednosti, raste s porastom doze SP. Pored toga, ono

ovisi i o vrsti podražaja. Uz jednake doze SP procentualno sniženje istosmjernih fluktuacija općenito je utoliko manje ukoliko je veći sadržaj »informacija« u primijenjenom senzornom podražaju.

(3) Kočenje kortikalnih stimulativnih procesa, koji dolaze do izraza u registrovanim crtežima istosmjernog potencijala, djelomično je zasnovano na direktnom djelovanju SP u moždanoj kori. Međutim, prema rezultatima usporednih mjerenja pri intraperitonealnoj i lokalnoj aplikaciji SP nije mogućno protumačiti ovo kočenje isključivo direktnim djelovanjem SP. Štaviše, sniženje retikularnog podražajnog nivoa predstavlja dalji bitan uzrok kočenja kortikalnih podražajnih procesa. Kako to pokazuje direktno registrovanje neuronskih izbijanja u retikularnoj formaciji moždanog debla, djelovanje SP znatno smanjuje i spontanu aktivnost i reakcije na podražaj u retikularnim strukturama.

Rezultati ovih istraživanja dopuštaju da se zaključi da kočenje stimulativnih procesa u retikulo-kortikalnom sistemu bitno sudjeluje u tvorbi poznatog sedativnog efekta SP.

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DISCUSSION

KRIVOY: Where were the electrodes located?

CASPERS: The d. c. potentials were led from various areas of the intact cortical surface and recorded against a reference point in the front portion of the muzzle which has proved sufficiently inactive.

v. EULER: Pernow and I have noted the effects of SP lasting for more than 1/2 hour after administration in the brain ventricles, indicating a slow inactivation.

CASPERS: Our observations on rats are in good agreement with your experimental results. We found that the direct cerebral effects produced by an i. p. injection of a medium dose of SP (cf. p. 37.) usually last as long as 30—60 min. after the administration. This finding, too, points to a slow inactivation of the substance.

STURMER: In terms of our highly purified SP, a dose 4,000 U./kg rat by injection corresponds to 120 μg/kg which cannot be regarded as a large dose.

Polypeptides like oxytocin, vasopressin or bradykinin are destroyed in a matter of minutes when injected. It is remarkable therefore that the effect of parenteral SP should last for about 0.5 hour.