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Behavioural and somatic effects of bradykinin injected into the cerebral ventricles of unanaesthetized rabbits

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1. The effects of bradykinin (1–5 μg) injected into the cannulated lateral cerebral ventricles were studied in unanaesthetized rabbits before and after intravenous atropine, diphemanil and morphine.

2. The intraventricular injections of bradykinin produced a short-lasting phase of behavioural excitation with vocalization followed by sedation. The behavioural excitation was associated with desynchronization in the electrocorticogram (e.co.g.), bradycardia and hypotension followed by tachycardia and hypertension. Tachypnoea was also observed. The subsequent phase of sedation was more prolonged and associated with synchronization of the e.co.g. and signs of catalepsy. Intense miosis was present during both phases.

3. With repeated intraventricular injections of bradykinin, excitation, miosis, cardiovascular responses and tachypnoea diminished and eventually disappeared but the sedation did not exhibit tachyphylaxis.

4. Atropine abolished the e.co.g. desynchronization, vocalization and bradycardia, reduced the duration of the excitatory and sedatory phase, diminished the tachycardia and hypotension, enhanced the hypertension, but did not affect the miosis and tachypnoea.

5. Diphemanil affected only the cardiovascular effects produced by intraventricular bradykinin. They were affected in the same way as by atropine.

6. Morphine did not affect the excitatory phase, but enhanced the cardiovascular effects produced by intraventricular bradykinin.

7. The intraventricular injection of bradykinin (50 μg) caused a reduction in the amount of noradrenaline but not of 5-hydroxytryptamine (5-HT) in the brain stem ; the amount of dopamine in the caudate nuclei was not affected.

8. It is suggested that central cholinergic and adrenergic systems are activated by intraventricular bradykinin.

In cats, different preparations of bradykinin give different results when injected into the cerebral ventricles. With the pure bradykinin prepared by Elliott, Horton & Lewis (1960) no effects were obtained (Lewis, 1963), whereas with the natural bradykinin, prepared by the action of snake venom (*B. jararaca*) on ox plasma (Rocha e Silva, Beraldo & Rosenfeld, 1949), as well as with the synthetic brady-

kinin (BRS-640, Sandoz) short-lasting excitation was produced followed by depression or sedation similar to that obtained with reserpine. Excitation was associated with desynchronization and depression with synchronization in the electroencephalogram (e.e.g.). From results obtained on the *encéphale isolé* and *cerveau isolé*, it appeared that the reticular formation was essential for the production of the desynchronization. The excitatory effects of the natural bradykinin were more pronounced than those of the synthetic polypeptide. With the synthetic compound the excitatory phase was, in fact, not regularly obtained (Corrado, Ramos & Rocha e Silva, 1960 ; Capek, 1963 ; Rocha e Silva, 1963 ; Capek, Corrado, Ferreira & Rocha e Silva, 1966 ; Graeff, Corrado, Pelá & Capek, 1967).

The difference in the action of natural and synthetic bradykinin is probably due to the presence in the natural preparation of a " bradykinin potentiating factor " (BPF) as an impurity and derived from the venom since, as shown by Ferreira (1965), such a factor is extracted from the venom by the same procedure as that used for the extraction of natural bradykinin. Another effect obtained in cats with intraventricular injection of bradykinin, when preceded by pre-treatment with BPF, was a reduction in the brain concentration of noradrenaline but not of 5-hydroxytryptamine (5-HT) (Graeff, Corrado, Pelá & Capek, 1967).

In rabbits, previous work has shown that injections of synthetic bradykinin into the cerebral ventricles produce behavioural and e.e.g. changes without requiring pre-treatment with BPF (Graeff, Corrado & Pelá, 1967). The present experiments are a continuation of this work. They deal with the effects produced by the injection of synthetic bradykinin injected into the cerebral ventricles of unanaesthetized rabbits. Effects on behaviour, the e.e.g., blood pressure and cardiac and respiratory rate were studied before and after intravenous injections of atropine, diphemanil or morphine. The behavioural effects were compared with those of intraventricular eledoisin, a polypeptide which, like bradykinin, dilates the intracranial vessels (Graeff, Ferreira, Corrado & Rocha e Silva, 1965). Finally, experiments were performed to find out if intraventricular injections of bradykinin influence the amounts of noradrenaline, 5-HT and dopamine in the rabbit brain.

Methods

Rabbits of both sexes weighing 2·5–3 kg were anaesthetized with nembutal (35 mg/kg intravenously) and placed in a Labtronics Model 4 Stereotaxic Instrument for implantation of a modified Collison cannula (Feldberg & Sherwood, 1953) into the lateral ventricles. The cannula consisted of a stainless steel needle measuring $12 \times 0{\cdot}8$ mm, open at both ends and with a small lateral outlet 1 mm above the lower end. The upper end was connected to a 10 mm PE-60 polyethylene tube. The skull was exposed, a burr hole was drilled 2 mm lateral to the sagital and 2 mm posterior to the coronary suture, and the cannula was inserted 7–8 mm deep, so that its tip rested in the lateral ventricle. The cannula was fixed to the bone with dental cement, and its correct position was ascertained at the end of each experiment by injecting 0·2 ml. methylene blue through the cannula and dissecting the brain.

Two or three days after implantation of the cannula, the animals were placed inside an $80 \times 80 \times 50$ cm box in a sound proof room to study behavioural effects. First, they were given intraventricular injections of 0·05 ml. artificial cerebrospinal fluid (c.s.f.) and observed for half an hour, and then either bradykinin (1 μg) or

eledoisin (10 μg) was injected and the animals were observed continuously for one or more hours.

To study the effects on the e.co.g., arterial blood pressure and respiration, brass cortical electrodes were screwed into both sides of the skull, one pair in the frontal, one in the parietal, and one in the occipital region immediately after implantation of the cannula into the lateral ventricle. The electrodes were fixed to the bone with dental cement; the animals were then removed from the stereotaxic instrument and the right common carotid artery was cannulated with PE-190 polyethylene tubing for recording the blood pressure. Six hours later, when the animals had recovered from the effects of the anaesthetic, they were placed in a restraining box inside the sound proof room. A 6-channel Grass Model 5 Polygraph served to record the e.co.g. The blood pressure was recorded by means of a Statham P23AC Pressure Transducer, and the spirogram by means of a Grass Model PT5A Volumetric Pressure Transducer. Heart and respiratory rates were taken from the records. As soon as the recordings showed that the animal was adapted to the new environment an intraventricular injection of 0·05 ml. of artificial c.s.f. was given. Intermittent recordings were taken during a control period of at least 30 min and two or three control alarm reactions were provoked by sounding a buzzer inside the sound proof room. After the drugs were injected, recordings and observations were made continuously for 10 minutes and then for 1 or 2 minutes every 5 minutes.

The brain monoamines were estimated in rabbits 2 or 3 days after implantation of the ventricular cannula. Twenty minutes after an intraventricular injection of either bradykinin or of 0·1 ml. artificial c.s.f., the rabbits were decapitated and exsanguinated under deep barbiturate anaesthesia. The brains were removed and dissected on ice. Noradrenaline and 5-HT were extracted separately from the brain stem (medulla, pons, mesencephalon and diencephalon), and assayed spectrofluorimetrically, the noradrenaline by the method of Shore & Olin (1958) and the 5-HT by that of Mead & Finger (1961). The dopamine was extracted from the caudate nuclei according to the method described by Brodie, Comer, Costa & Dlabac (1966) and revised by A. Cho & M. A. Beaven (personal communication). Both caudate nuclei were weighed, homogenized at 4° C with 40 times their weight of cold 0·4 N $HClO_4$ and centrifuged for 10 min at 650 *g*. An aliquot of 2·5 ml. of supernatant was then removed and added to 0·5 g of alumina and 5 ml. of Tris buffer 0·5 M pH 9. The mixture was gently shaken for 20 min and centrifuged at 650 *g* for 15 min. The supernatant was removed and the alumina washed six times with 5 ml. distilled water. After adding 2 ml. 0·1 N HCl to the aluminia, the tubes were shaken for 10 min and re-centrifuged. The dopamine was assayed fluorimetrically in 1 ml. of the acid supernatant according to Carlsson & Waldeck (1958) with a Zeiss Z FM 4C spectrofluorometer. The results were analysed statistically with Student's *t* test at a significance level of 0·05.

Drugs. Atropine sulphate, bradykinin (synthetic, BRS-640, Sandoz), eledoisin (synthetic, ELD-950, Sandoz), diphemanil (Prantal, Schering), morphine (hydrochloride, Merck). The doses given in the text refer to these preparations. Original solutions of BRS-640 and ELD-950 were evaporated *in vacuo* and redissolved in artificial c.s.f. before use. The pH of the solutions employed for intraventricular injection ranged between 7 and 8 and the volume injected was 0·05 ml. The composition of the artificial c.s.f. was that given by Elliott & Jasper (1949). Drugs injected intravenously were dissolved in 0·9% NaCl solution.

Results

Behavioural effects

With an intraventricular control injection of 0·05 ml. artificial c.s.f. no changes in behaviour were observed, but 1 or 2 min after an intraventricular injection of 1 μg bradykinin in 0·05 ml. of artificial c.s.f. there was increased alertness and an intense flight reaction. Vocalization occurred in five out of nine animals. The excitatory phase lasted 1 or 2 minutes and was followed by sedation which was maximal within 5 to 10 min and lasted 20 to 40 min. During this period spontaneous activity was decreased or absent, the rabbits were drowsy, there was ptosis and the ears dropped. The rabbits could, however, be woken up and still reacted to mechanical or auditory stimuli (changing position, clasping hands or buzzer). There was a certain degree of muscle rigidity with a tendency to assume a *hunched back* posture. If placed in uncomfortable or bizarre positions, such as placing the forelimbs at a higher level or a paw across the spine, the rabbits maintained these positions for several minutes, thus exhibiting signs of catalepsy. Strong miosis was present throughout the excitatory and sedative phase. With repeated intraventricular injections of bradykinin (1 μg) at hourly intervals the signs of excitation and the miosis disappeared with the second or third injection. Excitation was not obtained even after a 24 hr interval unless the dose of bradykinin was increased to 5 μg. On the other hand, there was little or no tendency to tachyphylaxis as far as sedation was concerned.

Eledoisin (10 μg) given intraventricularly caused an immediate alert reaction without vocalization ; this reaction consisted of increased exploratory activity and psychomotor excitation similar to that evoked by amphetamine. It lasted for more than 2 hr with gradual but complete recovery.

Circulatory, respiratory and e.co.g. effects

An intraventricular injection of 0·05 ml. artificial c.s.f. did not affect circulation, respiration, or the electrical activity of the cerebral cortex. Thirty–sixty sec after an intraventricular injection of bradykinin (1–5 μg), however, there was a sudden fall in arterial blood pressure with bradycardia followed after 10–20 sec by a rise of 16·4% beyond the pre-injection level and tachycardia, which amounted to a mean increase of 20·4% and lasted 1 min or less. In addition, the rate of respiration increased by 19 to 25·1%, as shown in Table 1. Vocalization occurred in five out of seven animals. On the e.co.g. there was first desynchronization coinciding with the circulatory and respiratory changes as well as with the excitatory phase followed after 3 to 5 min by waves of high amplitude and an increase in the number of spindles. This change persisted throughout the sedation phase. These changes are summarized in Fig. 1.

On repeated injections of bradykinin (5 μg) at 40 min intervals there was disappearance not only of the excitatory phase but also of the bradycardia, the blood pressure effects, respiratory changes and desynchronization of the e.co.g. In the latter, however, the waves of high amplitude and the increase in the number of spindles were still obtained. Fig. 2 shows the diminution and disappearance of bradycardia with four successive injections of bradykinin (5 μg).

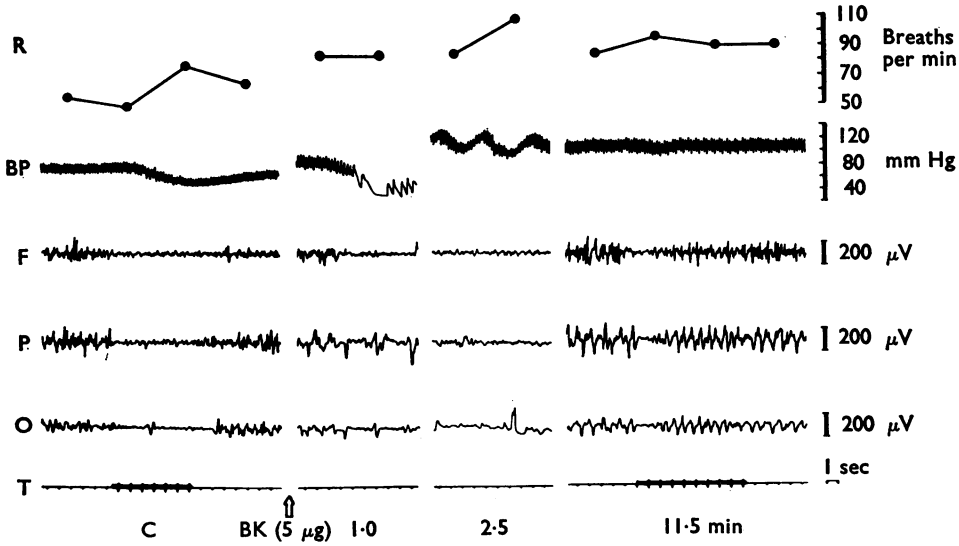

FIG. 1. Unanaesthetized rabbit, 3·0 kg. Intraventricular injection of bradykinin (BK). R, Respiration rate; BP, blood pressure; F, P and O, respectively bilateral frontal, parietal and occipital e.co.g. tracings; T, time. The full time-line indicates an alarm reaction to a buzzer. C, control tracings before and subsequent panel at 1, 2·5 and 11·5 min after bradykinin administration.

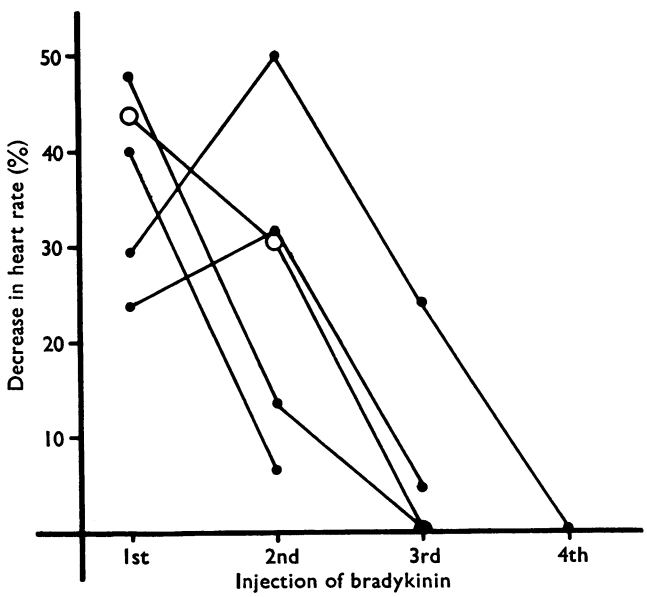

FIG. 2. Decrease of the bradycardia in five rabbits with repeated intraventricular injections of 5 μg (●) or 2·5 μg (○) of bradykinin. Time-interval between two successive injections, 40 min. Each point represents an individual value.

Atropine pre-treatment

An intraventricular injection of bradykinin (5 μg) 30 min after an intravenous injection of atropine 2 mg/kg still produced miosis and elicited both the excitatory and inhibitory phase, but the duration of both phases was reduced. No vocalization, however, occurred in any of the four rabbits in which the bradykinin was injected after atropine. The heart rate was increased by the atropine injection but the effects of bradykinin were diminished or absent. There was an insignificant bradycardia and the subsequent tachycardia amounted to 12·9% compared with 20·4% in the animal which did not receive atropine. The initial hypotension was also reduced but the subsequent hypertension was enhanced. The tachypnoea produced by the bradykinin injection was not affected by the atropine which itself had produced a slight acceleration of respiration. The differences are summarized in Table 1. Atropine is known to produce in the e.co.g. increased synchronization and increased spindle frequency, with blockade of arousal reaction to external stimuli though with persistency of behavioural excitation (Wikler, 1952; Rinaldi & Himwich, 1955; Longo, 1956). This effect was also observed in the present experiments. After atropine the bradykinin no longer produced desynchronization of the e.co.g., but the sedation induced by bradykinin was shortened.

Diphemanil pre-treatment

To avoid the cardiac dysrhythmia caused by a rapid intravenous injection of diphemanil a 2 mg/ml. solution was infused at a rate of 1 ml./min into the marginal vein of an ear until the cardiovascular effects of 20 μg/kg of acetylcholine were found to be abolished. This happened after the infusion of 4–8 mg/kg. Infused in

TABLE 1. *Behavioural and cardio-respiratory effects of the intraventricular injection of bradykinin and its interaction with atropine, diphemanil and morphine in the unanaesthetized rabbit*

Treatment	Number of animals	Blood pressure % Initial response	Blood pressure % Late response	Heart rate % Initial response	Heart rate % Late response	Respiratory rate %	Excitation phase min (mean ±S.D.)	Sedation phase min (mean ±S.D.)
BK 1 μg	6	−39·2	+1·0	−20·4	+9·8	+19·0	1·4±1·2	13·0±7·6
BK 5 μg	7	−39·3	+16·4	−36·4	+20·4	+25·1	2·8±1·4	32·1±4·9
Atropine +BK 5 μg	4	−20·3	+24·6	−0·2	+12·9	+25·9	1·9±0·6	25·0±4·1
Diphemanil +BK 5 μg	5	−25·0	+56·9	−5·4	+35·4	+30·5	2·3±0·5	35·0±8·2
Morphine +BK 5 μg	4	−35·7	+23·1	−53·1	+27·2	+48·9	2·8±0·8	—

Each figure for blood pressure, heart rate or respiratory rate indicates the percentage change in the mean value produced by intraventricular bradykinin (BK). Atropine (2 mg/kg), diphemanil (4–8 mg/kg) and morphine (10 mg/kg) were injected intravenously.

TABLE 2. *Effects of bradykinin (50 μg) on noradrenaline and 5-HT content of brain stem and on dopamine content of caudate nuclei 20 min after its intraventricular injection into unanaesthetized rabbits*

Amine	Concentration (μg) (mean±S.E.M.) Control	Bradykinin
Noradrenaline	0·95±0·09 (6)	0·64±0·06* (6)
5-Hydroxytryptamine	0·77±0·06 (5)	0·84±0·07 (5)
Dopamine	5·53±0·32 (4)	5·44±0·81 (4)

Results expressed in μg/g fresh brain tissue and corrected for mean recovery of 70% noradrenaline, 69% 5-HT and 82% dopamine. Figures in parentheses refer to number of animals. * Indicates significance at 0·05 level according to the *t* test.

this way in five rabbits, diphemanil produced a tendency for the arterial blood pressure to fall and for the respiration rate to increase. The heart rate fell in one rabbit and rose in another, but did not change much in the remaining three animals. The infusion produced no changes in behaviour or in the electrical activity of the cerebral cortex.

Twenty minutes after the diphemanil infusion an intraventricular injection of bradykinin (5 μg) produced the same or slightly different changes in behaviour, e.co.g. and respiration as in control rabbits. Miosis occurred in all and vocalization in three of the five rabbits. The cardiovascular effects, however, were affected in the same way as after atropine. There was an insignificant bradycardia and the initial hypotension was attenuated whilst the subsequent tachycardia and hypertension were enhanced, as shown in Table 1.

Morphine pre-treatment

In four rabbits the effects of intraventricular injections of bradykinin (5 μg) were examined 30 min after an intravenous injection of morphine (10 mg/kg) which had produced deep sedation, synchronization of the e.co.g. and slowing of respiration but no cardiovascular changes. The bradycardia and tachypnoea produced by the bradykinin were enhanced but the initial hypotension was the same as in control animals. The tachycardia and the late hypertension were slightly increased.

Although the animals were deeply sedated by the morphine the bradykinin injection produced excited behaviour as in controls, consisting of an intense flight reaction, vocalization in two out of four rabbits and desynchronization of the e.co.g. Miosis occurred in all four, as in the controls.

Effect on monoamine content of brain

Twenty minutes after an intraventricular injection of bradykinin (50 μg) there was a significant fall in the noradrenaline content of the brain stem whereas its 5-HT content showed a tendency to increase. The increase, however, was not significant at a level of $P<0{\cdot}05$. The dopamine content of the caudate nucleus was not significantly affected by the injection. The results are summarized in Table 2.

Discussion

Many of the effects obtained in the present experiments with bradykinin injected into the cerebral ventricles resemble those obtained previously by other authors on its injection into the cisterna magna (Bertolini, Mucci & Ferrari, 1966; Sicuteri, Franchi, Del Bianco & Fanciullacci, 1967). This similarity suggests that these effects are due to an action on structures reached from the subarachnoid space since drugs injected into the cerebral ventricles pass into it, but drugs injected into the cisterna do not enter the cerebral ventricles (Domer & Feldberg, 1960; Banerjee, Burks, Feldberg & Goodrich, 1968).

The initial behavioural excitation with vocalization, hyperventilation and biphasic cardiovascular changes which is similar to the pseudo-affective reactions of Sherrington (1947) appears to be more pronounced in rabbits than in cats in which it has not been regularly obtained with intraventricular injections of bradykinin and then only with larger doses (Corrado *et al.*, 1960; Capek *et al.*, 1966; Graeff,

Corrado & Pelá, 1967). In cats and dogs a strong pseudo-affective reaction is, however, obtained with bradykinin injected into the carotid artery and is attributed to stimulation of intracranial vascular pain receptors because it is abolished by morphine (Guzman, Braun & Lim, 1962). A different site of action is postulated for the pseudo-affective reaction of intraventricular bradykinin since this response was not abolished by morphine but reduced by atropine, which has no strong analgesic action. It is also unlikely that the pseudo-affective reaction to intraventricular bradykinin is due to dilatation of the cerebral vessels since eledoisin which dilates the cerebral vessels to the same extent as bradykinin (Carpi & Corrado, 1961 ; Graeff *et al.*, 1965) did not produce a pseudo-affective reaction.

In rabbits, Bertolini *et al.* (1966) also obtained a pseudo-affective response on injection of bradykinin (in doses greater than 2·5 to 5 μg) into the cisterna magna and Sicuteri *et al.* (1967) described " squeak and flight behaviour " in response to such injections. They attributed the response to an interaction of bradykinin with pain receptors because they found that it was abolished by indomethacin. In unpublished experiments we obtained a similar effect with indomethacin in that it reduced or abolished the excitatory phase of intraventricular bradykinin. However, it also reduced the sedation phase and moreover pentazocine did not change the behaviour pattern of intraventricular bradykinin. These findings together with the results obtained in the present experiments with morphine and atropine make it unlikely that the pseudo-affective reaction obtained not only on intraventricular but also on intracisternal injection of bradykinin is a simple reaction to pain.

The bradycardia produced by intraventricular bradykinin is of vagal origin since it is abolished by atropine and diphemanil, but the hypotension results probably mainly from inhibition of sympathetic vasomotor tone since it was little affected by atropine. We have no explanation for the observed fact that the intense miosis produced by the intraventricular injection of bradykinin, was not abolished by atropine nor by diphemanil.

Central cholinergic and adrenergic pathways may be involved in the responses to intraventricular bradykinin. The effect of atropine and the reduction in noradrenaline content of the brain stem may signify the involvement of central cholinergic and adrenergic neurones in the response to intraventricular bradykinin. From the inhibitory effect obtained with atropine and atropine-like substances on the expression of conditioned defensive behaviour (or emotional fear reaction) in cats and dogs, Ilyutchenok (1968) postulated the involvement of central cholinergic synapses which are blocked by atropine. On the other hand, atropine and scopolamine do not inhibit the flight reaction to electrical stimulation of the hypothalamus (Longo, 1956). The complexity of such cholinergic mechanisms in the activation of the reticular formation in the midbrain is well known and is reviewed by Marczynski (1967). More recently, Beaulnes & Ling (1968) found that bradykinin injected intraventricularly increases the acetylcholine content of the brain.

According to Reis, Miura, Weinbren & Gunne (1967), central adrenergic neurones are activated in the expression of pseudo-affective reactions and in drug induced excitation. Gunne & Lewander (1966) found in cats, that electrical stimulation of the lateral hypothalamus and amygdala which resulted in rage behaviour lead to a significant decrease in the noradrenaline content of the brain without significant changes in the level of 5-HT or dopamine. This finding corresponds to the effect of intraventricular bradykinin on the monoamine levels of the rabbit brain. If the

bradykinin-induced excitation requires activation of central adrenergic mechanisms lack of the transmitter in these neurones could account for the tachyphylaxis. It could also account for the behavioural sedation and tranquillization produced by bradykinin. The same explanation has been put forward to account for the tranquillizing action of reserpine (Carlsson, Lindqvist & Magnusson, 1957; Carlsson, Rosengren, Bertler & Nilsson, 1957; Costa & Brodie, 1964) and of α-methyl-tyrosine (Spector, Sjoerdsma & Udenfriend, 1965).

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REFERENCES

BANERJEE, U., BURKS, T. F., FELDBERG, W. & GOODRICH, C. A. (1968). Temperature effects and catalepsy produced by morphine injected into the cerebral ventricles of rabbits. *Br. J. Pharmac. Chemother.*, **33**, 544–551.

BEAULNES, A. & LING, G. M. (1968). Effect of angiotensin and bradykinin on brain acetylcholine. *Fedn Proc.*, **27**, 471.

BERTOLINI, A., MUCCI, P. & FERRARI, W. (1966). La bradichinina come possibile agente causale dell'emicrania. III. Effetto dell'iniezione endocisternale di bradichinina nel cane e nel coniglio. *Boll. Soc. ital. Biol. sper.*, **42**, 1922–1925.

BRODIE, B. B., COMER, M. S., COSTA, E. & DLABAC, A. (1966). The role of brain serotonin in the mechanism of the central action of reserpine. *J. Pharmac.*, **152**, 340–349.

CAPEK, R. (1963). Some effects of bradykinin on the central nervous system. In *First International Pharmacological Meeting*, vol. 9, part 1: *Bradykinin and Vaso-Dilating Polypeptides*, ed. Rocha e Silva, M. & von Euler, U. S., pp. 61–65. Oxford: Pergamon Press.

CAPEK, R., CORRADO, A. P., FERREIRA, S. H. & ROCHA E SILVA, M. (1966). The effect of bradykinin on the electroencephalogram. *Activitas nerv. sup.*, **8**, 419–420.

CARLSSON, A., LINDQVIST, M. & MAGNUSSON, T. (1957). 3-4-Dihydroxyphenylalanine and 5-hydroxytryptophan as reserpine antagonists. *Nature, Lond.*, **180**, 1200.

CARLSSON, A., ROSENGREN, E., BERTLER, A. & NILSSON, J. (1957). Effect of reserpine on the metabolism of catecholamines. In *Psychotropic Drugs*, ed. Garattini, S. & Ghetti, V., pp. 363–372. Amsterdam: Elsevier Publishing Company.

CARLSSON, A. & WALDECK, B. (1958). A fluorimetric method for the determination of dopamine (3-hydroxytyramine). *Acta physiol. scand.*, **44**, 293–298.

CARPI, A. & CORRADO, A. P. (1961). Cerebral vascular action of bradykinin in the dog. *Experientia*, **17**, 326–329.

CORRADO, A. P., RAMOS, A. O. & ROCHA E SILVA, M. (1960). On possible central effects of bradykinin in the cat. *Acta physiol. latinoamer.*, **9**, 222.

COSTA, E. & BRODIE, B. B. (1964). Concept of the neurochemical transducer as an organized molecular unit at sympathetic nerve endings. In *Progress in Brain Research*, vol. 8, ed. Himwich, H. E. & Himwich, W. A., pp. 168–185. Amsterdam: Elsevier Publishing Company.

DOMER, F. R. & FELDBERG, W. (1960). Some central actions of adrenaline and noradrenaline when administered into cerebral ventricles. In *Adrenergic Mechanisms*, ed. Vane, J. R., pp. 386–392. London: Churchill.

ELLIOTT, D. F., HORTON, E. W. & LEWIS, G. P. (1960). Actions of pure bradykinin. *J. Physiol., Lond.*, **153**, 473–480.

ELLIOTT, K. & JASPER, H. H. (1949). Physiological salt solutions for brain surgery; studies of local *p*H and pial vessel reactions to buffered and unbuffered isotonic solutions. *J. Neurosurg.*, **6**, 140.

FELDBERG, D. & SHERWOOD, S. L. (1953). A permanent cannula for intraventricular injections in cats. *J. Physiol., Lond.*, **120**, 3*P*.

FERREIRA, S. H. (1965). A bradykinin-potentiating factor (BPF) present in the venom of *Bothrops jararaca*. *Br. J. Pharmac. Chemother.*, **24**, 163–169.

GRAEFF, F. G., CORRADO, A. P. & PELÁ, I. R. (1967). Efeitos comportamentais e eletroencefalográficos da bradicinina. Potenciação pelo FPB. *Ciência e Cultura*, **19**, 399.

GRAEFF, F. G., CORRADO, A. P., PELÁ, I. R. & CAPEK, R. (1967). Actions of bradykinin upon the central nervous system. In *Int. Symposium on Vaso-Active Polypeptides: Bradykinin and Related Kinins*, ed. Rocha e Silva, M. & Rothschild, H. A., pp. 97–102. São Paulo: Edart.

GRAEFF, F. G., FERREIRA, S. H., CORRADO, A. P. & ROCHA E SILVA, M. (1965). Potentiation of the cerebral vascular action of bradykinin by the "bradykinin potentiating factor" (BPF) in the dog. *Experientia*, **21**, 607–611.

GUNNE, L. M. & LEWANDER, T. (1966). Monoamines in brain and adrenal glands of cats after electrically induced defense reaction. *Acta physiol. scand.*, **67**, 405–410.

GUZMAN, F., BRAUN, C. & LIM, R. K. S. (1962). Visceral pain and the pseudoaffective response to intraarterial injection of bradykinin and other algesic agents. *Archs int. Pharmacodyn. Thér.*, **136**, 353–384.

ILYUTCHENOK, R. Y. (1968). Cholinergic brain mechanisms and behaviour. *Progress in Brain Research*, vol. 28, ed. Bradley, P. B. & Fink, M., pp. 134–148. Amsterdam: Elsevier Publishing Company.

LEWIS, G. P. (1963). In *First International Pharmacological Meeting*, vol. 9, part 1: *Bradykinin and Vaso-Dilating Polypeptides*, ed. Rocha e Silva, M. & von Euler, U. S., p. 65. Oxford: Pergamon Press.

LONGO, V. G. (1956). Effects of scopolamine and atropine on EEG and behavioural reactions due to hypothalamic stimulation. *J. Pharmac. exp. Ther.*, **116**, 198–208.

MARCZYNSKI, T. J. (1967). Topical application of drugs to subcortical brain structures and selected aspects of electrical stimulation. *Ergeb. der Physiologie*, **59**, 86.

MEAD, J. A. & FINGER, K. F. (1961). A simple extraction method for the determination of both norepinephrine and serotonin in brain. *Biochem. Pharmac.*, **6**, 52–53.

REIS, D. J., MIURA, M., WEINBREN, M. & GUNNE, L. M. (1967). Brain catecholamines: Relation to defense reaction evoked by acute brain stem transection in cat. *Science, N.Y.*, **156**, 1768–1770.

RINALDI, F. & HIMWICH, H. E. (1955). Alerting responses and the actions of atropine and cholinergic drugs. *A.M.A. Arch. Neurol. Psychiat.*, **73**, 387–395.

ROCHA E SILVA, M. (1963). The physiological significance of bradykinin. *Ann. N.Y. Acad. Sci.*, **104**, 190–211.

ROCHA E SILVA, M., BERALDO, W. T. & ROSENFELD, G. (1949). Bradykinin, a hypotensive and smooth muscle stimulating factor released from plasma globulin by snake venoms and trypsin. *Am. J. Physiol.*, **156**, 261–273.

SHERRINGTON, C. S. (1947). *The Integrative Action of the Nervous System*, 7th ed., p. 252. Cambridge: Cambridge University Press.

SHORE, P. A. & OLIN, J. (1958). Identification and chemical assay of norepinephrine in brain and other tissues. *J. Pharmac. exp. Ther.*, **122**, 295–300.

SICUTERI, F., FRANCHI, G., DEL BIANCO, P. L. & FANCIULLACCI, M. (1967). Peptides and pain. In *International Symposium on Vaso-Active Polypeptides: Bradykinin and Related Kinins*, ed. Rocha e Silva, M. & Rothschild, H. A., pp. 251–261. São Paulo: Edart.

SPECTOR, S., SJOERDSMA, A. & UDENFRIEND, S. (1965). Blockade of endogenous NE synthesis by α-methyl-tyrosine, an inhibitor of tyrosine hydrolylase. *J. Pharmac. exp. Ther.*, **147**, 86–95.

WIKLER, A. (1952). Pharmacologic dissociation of behaviour and EEG "sleep patterns" in dogs: Morphine, N-allylnormorphine and atropine. *Proc. Soc. exp. Biol. Med.*, **79**, 261–265.

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