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Pharmacological modifications of sympathetic responses elicited by hypothalamic stimulation in the rat

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1. The rise in blood pressure and the eyelid contractions elicited by electrical stimulation of the posterior hypothalamus in anaesthetized rats were studied for the assessment of drug effects on the sympathetic system. They were compared with the noradrenaline pressor effect and eyelid responses to cervical sympathetic nerve stimulation.

2. Phentolamine caused a similar reduction of the pressor responses induced by hypothalamic stimulation and by noradrenaline. It also reduced the eyelid contractions elicited by central and by peripheral electrical stimulation.

3. Guanethidine caused an immediate inhibition of the pressor response to hypothalamic stimulation, while it potentiated noradrenaline effects. Eyelid contractions elicited both by central and by peripheral electrical stimulation were inhibited.

4. Chlorpromazine inhibited the pressor responses both to hypothalamic stimulation and to noradrenaline, but it caused a much greater reduction in the centrally evoked eyelid responses than in those due to peripheral stimulation.

5. Diazepam caused a reduction of sympathetic responses to central stimulation but not to peripherally elicited responses.

6. In unanaesthetized rats the rise in blood pressure induced by hypothalamic stimulation was accompanied by increased locomotor activity culminating in a flight reaction. In contrast to the pressor effect, which was reduced by all four of the above-mentioned drugs, the flight reaction was not affected by phentolamine and guanethidine and only delayed by chlorpromazine and diazepam at dose levels which impaired the spontaneous locomotor activity.

In a preliminary paper (Morpurgo & Morillo, 1962) we suggested that the simultaneous recording of different sympathetic responses elicited by hypothalamic stimulation would provide a better procedure for the evaluation of drugs acting on the autonomic nervous system than procedures involving the stimulation of single organs.

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We have studied the drug-induced modifications of pressor responses and nictitating membrane contractions elicited by stimulation of the posterior hypothalamus in the cat. Because of important species differences in responses to drugs, it was considered worth while to extend the investigation to the rat, which is more commonly used in the study of drugs acting on the central nervous system. The use of the same animal species seems particularly important if one wants to correlate drug-induced autonomic and behavioural changes. Moreover, the use of small animals and the availability of more homogeneous experimental subjects presented additional advantages.

Since we observed that the electrical stimulation of the posterior hypothalamus of the rat induced an immediate, conspicuous opening of the palpebral fissure, we have recorded the eyelid contractions simultaneously with the pressor responses. In an attempt to differentiate between peripheral and central effects of drugs, we have compared the influence of substances on the eyelid contractions induced by hypothalamic stimulation and by stimulation of the cervical sympathetic nerve. The peripheral adrenolytic activity of the compounds was assessed on the modifications of the pressor responses to exogenous noradrenaline.

Most of the experiments were performed on anaesthetized rats ; in some other experiments we have studied the behavioural reactions and the pressor responses to hypothalamic stimulation in unanaesthetized, free-moving rats with chronically implanted electrodes and permanent cannulation of the aorta.

For an evaluation of the technique, we have studied the effects of some well-known compounds, representatives of different pharmacological classes. In this paper are reported the results obtained with an adrenolytic agent (phentolamine), a sympathetic nerve blocking drug (guanethidine), a major tranquillizer with important effects on the autonomic nervous system (chlorpromazine) and with a tranquillizer having only mild peripheral autonomic effects (diazepam), which has been shown to reduce pressor responses to hypothalamic stimulation in the cat (Schallek, Zabransky & Kuehn, 1964).

Methods

Male albino Wistar rats, 250–300 g body weight, were used throughout these studies.

Experiments on anaesthetized rats

The rats were anaesthetized with urethane (1.3 g/kg intraperitoneally) ; a carotid artery and a jugular vein were cannulated with polyethylene tubings, the arterial cannula being filled with a 2% solution of the anticoagulant G 31 150 (active ingredient of Hemeran, J. R. Geigy S.A., Basle). Body temperature was maintained at 36.5° C by means of a heat lamp connected to a rectal thermometer.

The animal's head was fixed in a Stoelting stereotaxic apparatus, and a monopolar stainless steel electrode (0.5 mm diameter, 0.5 mm uninsulated tip) was introduced through a small trephine hole at the co-ordinates F 4.6, L 0.5, H −2.5 according to De Groot's atlas (1963), corresponding to the posterior hypothalamus ; the indifferent electrode was attached to the ear bar.

In order to stimulate the cervical sympathetic nerve, the head of the rat was immobilized in a head holder, the nerve was dissected, placed over shielded platinum

electrodes and embedded in solid paraffin. When alternate central and peripheral stimulation was to be performed on the same animal, the hypothalamic electrode was secured to the skull with dental cement.

The following parameters were recorded on an Offner type S dynograph: carotid blood pressure via a Statham P 23 Db pressure transducer; heart rate by means of subcutaneous electrodes using the EKG-tachograph channel; eyelid contractions with Grass force displacement transducers FT 0.03 C.

Electrical stimulation was made through a Grass model SD 5 stimulator, with stimulus-isolation unit. Unless otherwise specified, square wave pulses of 1.5 msec duration at 70 c/s were used for hypothalamic stimulation, and of 2.5 msec duration at 25 c/s for nerve stimulation, for periods of 5 sec. The stimulus intensity varied from 2 to 5 V. The current flow was monitored on an amperemeter. Humoral stimulation was obtained with intravenous noradrenaline hydrochloride (Arterenol®, Hoechst), in a saline solution containing ascorbic acid 100 μg/ml. A dose was selected which produced a pressor response of approximately the same magnitude as that elicited by hypothalamic stimulation.

Experiments on unanaesthetized rats

Rats were anaesthetized with pentobarbital sodium (50 mg/kg intraperitoneally); a cannula was implanted in the aorta according to the procedure described by Popovic & Popovic (1960). The stimulating electrode, stereotaxically implanted in the posterior hypothalamus, and a cranial indifferent electrode were sealed with dental cement. At least 24 hr were allowed for recovery from anaesthesia before starting the experiments.

After flushing the arterial cannula with diluted heparin solution, continuous blood pressure was recorded in unrestrained rats. In some experiments the locomotor activity and the flight reaction induced by hypothalamic stimulation were registered on the dynograph by means of a Grass displacement transducer connected to the exteriorized arterial cannula. In further experiments, behavioural reactions were recorded on Video tape in a closed circuit television apparatus.

Drugs

The following preparations were employed: Regitine® (ampoules, Ciba); guanethidine sulphate (active ingredient of Ismelin®, Ciba); Largactil® (ampoules, Specia) and Valium® (ampoules, Roche).

In order to avoid abrupt cardiovascular changes caused by intravenous injections, the test drugs were administered by intraperitoneal route, in a volume of 2 ml./kg.

Results

Electrical stimulation of the posterior hypothalamus in anaesthetized rats induced an immediate blood pressure rise, usually accompanied by a reduction of the heart rate, and a conspicuous bilateral opening of the palpebral fissures, due to the retraction of both superior and inferior eyelids. Although similar reactions could be elicited by stimulation of other diencephalic and mesencephalic structures, optimal responses were obtained when the stimulating electrode was aimed at the nucleus dorsomedialis hypothalami. Graded responses were obtained by increasing the frequencies of stimulation; maximal responses were observed in the range

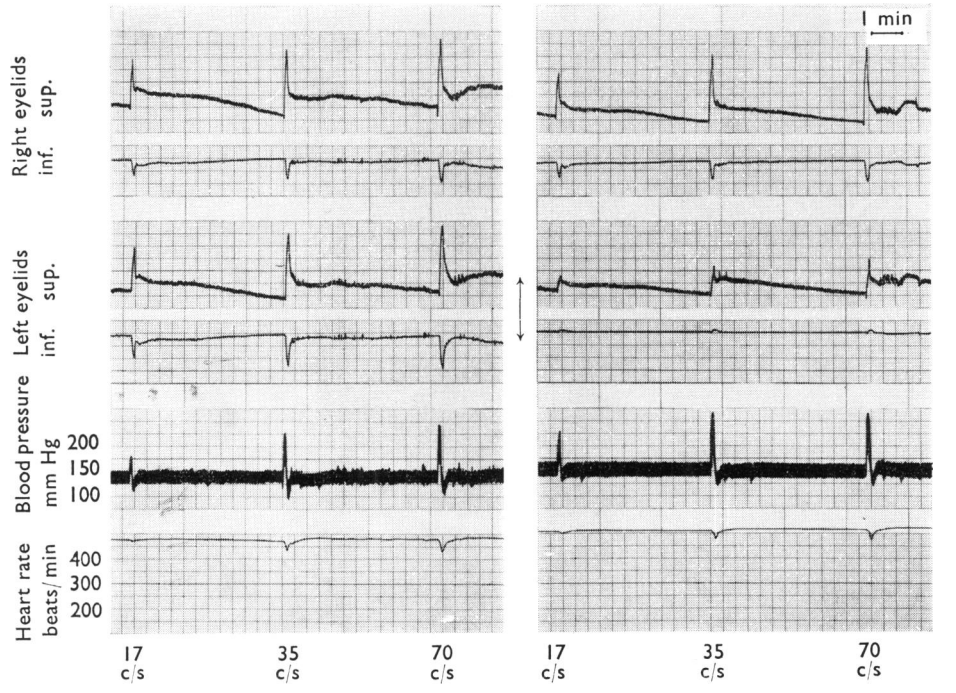

FIG. 1. Effect of sectioning the left cervical sympathetic nerve (↕) on the eyelid contractions elicited by hypothalamic stimulation (4 V; varying frequencies) in anaesthetized rat. In this and following records, contractions of the inferior eyelid are represented by downward deflections.

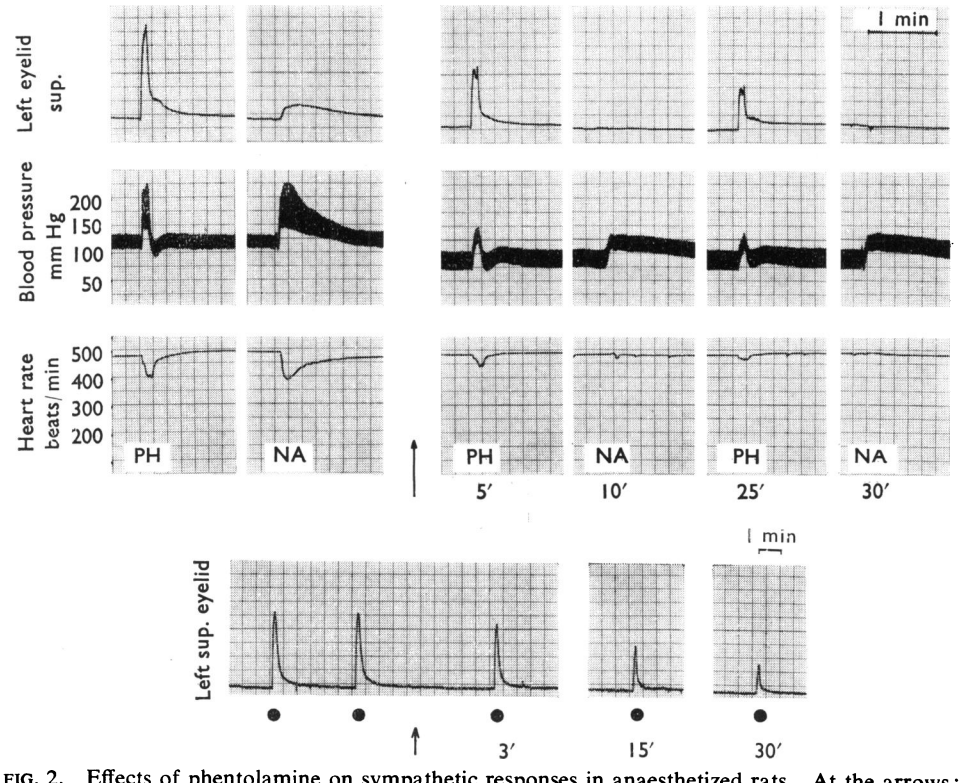

FIG. 2. Effects of phentolamine on sympathetic responses in anaesthetized rats. At the arrows: phentolamine 5 mg/kg i.p. Upper tracings: PH=hypothalamic stimulation (4.5 V); NA=noradrenaline (5 μg i.v.). Lower tracing: at dots, stimulation of the left cervical sympathetic nerve (4 V).

TABLE 1. *Modifications of centrally and peripherally evoked sympathetic responses in anaesthetized rats*

Drug	Dose (mg/kg i.p.)	Mean absolute changes of basal cardiovascular values		Mean percentage changes			
				Pressor responses		Superior eyelid contractions	
		Systolic blood pressure (mm Hg)	Heart rate (beats/min)	Hypothalamic stimulation	Noradrenaline	Hypothalamic stimulation	Cervical sympathetic n. stimulation
Phentolamine	5	−20±5 (13)	−7±15 (11)	−64±7 (9)	−59±4 (9)	−49±8 (9)	−57±10 (7)
Guanethidine	5	−12±4 (14)	+13±16 (10)	−93±4 (5)	>+42±4 (10)	−69±11 (5)	−66±8 (10)
Chlorpromazine	5	−32±4 (16)	−98±18 (14)	−76±7 (8)	−77±5 (10)	−76±4 (8)	−13±4 (11)
Diazepam	5	−8±4 (14)	−25±12 (11)	−39±6 (9)	+8±10 (7)	−46±6 (10)	0±2 (4)

Changes calculated approximately 30 min after drug injections in comparison with the corresponding pre-drug control values. The number of experiments is shown in parenthesis. Mean control values: systolic blood pressure: 111±3 mm Hg (N=57); heart rate: 391±9 beats min (N=46); hypothalamic pressor response: +81±4 mm Hg (N=31); noradrenaline pressor response: +94±4 mm Hg (N=36).

between 50 and 100 c/s. Both the pressor effect and the eyelid contractions immediately subsided at the end of the period of stimulation. The eyelid responses closely resembled those induced by stimulation of the sympathetic cervical nerve, as they could be demonstrated in the experiments with alternate central and peripheral stimulation. In some experiments equal contractions of the superior eyelid could be elicited by hypothalamic and sympathetic nerve stimulation; in other experiments, however, the eyelid responses to hypothalamic stimulation were reduced on the side of nerve stimulation, probably due to mechanical damage of the nerve caused by the peripheral electrodes. The importance of the sympathetic outflow for the eyelid response to hypothalamic stimulation was assessed by making a unilateral section of the cervical sympathetic nerve. In five experiments $71 \pm 4\%$ inhibition of the left superior eyelid and 100% inhibition of the inferior eyelid con-

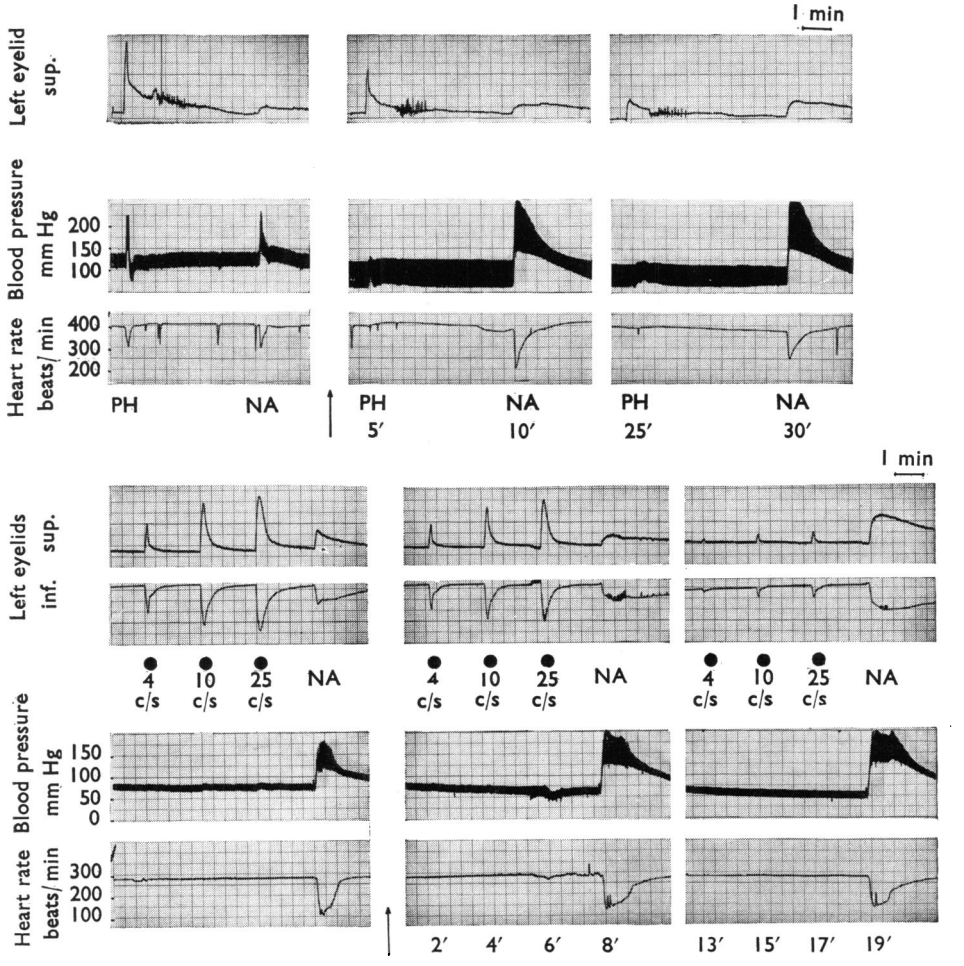

FIG. 3. Effects of guanethidine on sympathetic responses in anaesthetized rats. At the arrows: guanethidine 5 mg/kg i.p. Upper tracings: PH=hypothalamic stimulation (2.5 V); NA=noradrenaline (2.5 μg i.v.). Lower tracings: at dots, stimulation of the left cervical sympathetic nerve (3 V; varying frequencies); NA=noradrenaline (10 μg i.v.).

tractions induced by hypothalamic stimulation were observed after sectioning the left cervical sympathetic nerve, while the contralateral responses appeared unchanged (Fig. 1).

Table 1 summarizes the results obtained with four drugs given intraperitoneally at a dose of 5 mg/kg. The evaluation was based on the responses recorded about 30 min after administration, when the effects were maximal. In control experiments all the responses showed minimal variations during several hours of observation.

Phentolamine caused a similar reduction of the pressor responses induced by hypothalamic stimulation and by noradrenaline, and an inhibition of the eyelid contractions induced both by peripheral and by central stimulation (Fig. 2). The time course of this inhibiting activity was approximately the same for all the responses.

Guanethidine caused an immediate blockade of the pressor response induced by hypothalamic stimulation and a marked potentiation of the noradrenaline hypertensive effect. The eyelid contractions induced by electrical stimulation either of the hypothalamus or of the cervical sympathetic nerve were inhibited ; however, the

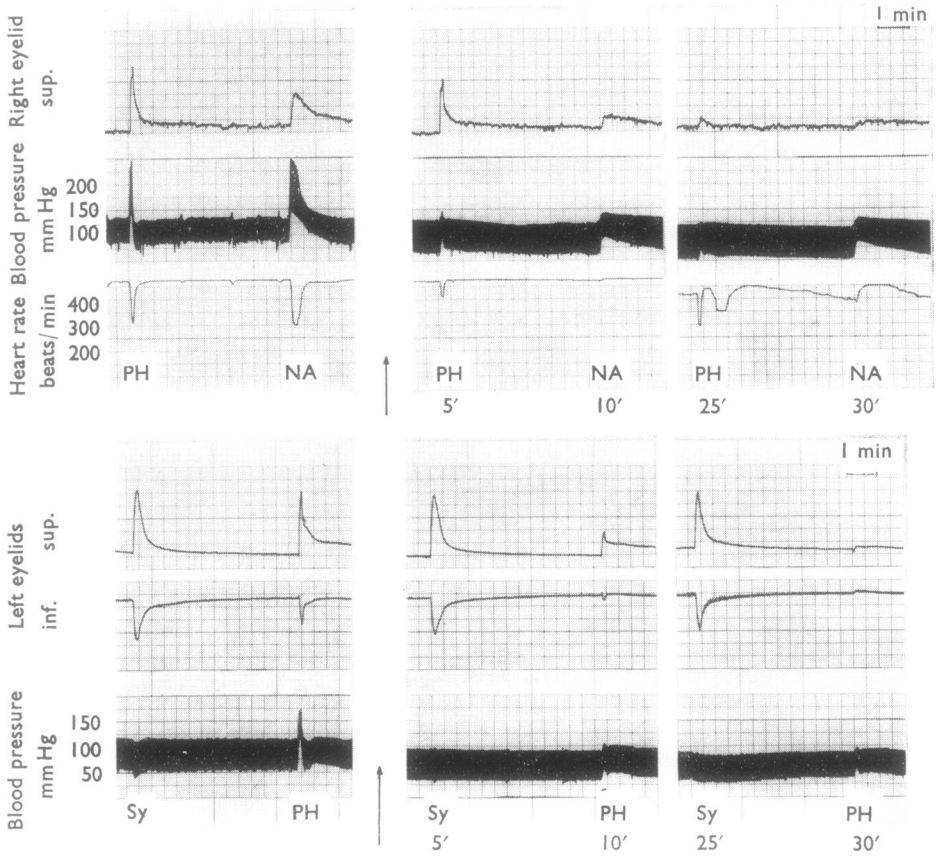

FIG. 4. Effects of chlorpromazine on sympathetic responses in anaesthetized rats. At the arrows: chlorpromazine 5 mg/kg i.p. Upper tracings: PH=hypothalamic stimulation (3.5 V); NA=noradrenaline (5 μg i.v.). Lower tracings: Sy=stimulation of the left cervical sympathetic nerve (3.5 V); PH=hypothalamic stimulation (3 V).

onset of the inhibition was delayed in comparison with the effects on the blood pressure (Fig. 3).

Chlorpromazine caused an immediate blockade of the pressor responses elicited both by hypothalamic stimulation and by noradrenaline and a somewhat delayed inhibition of the eyelid responses to hypothalamic stimulation; the eyelid response to stimulation of the cervical sympathetic nerve was scarcely affected (Fig. 4). Even a large dose (20 mg/kg) caused a relatively small inhibition ($32 \pm 4\%$; $N=5$) of the eyelid contraction elicited by peripheral electrical stimulation.

Diazepam caused a reduction of the sympathetic responses elicited by hypothalamic stimulation, but did not inhibit, even in high doses, the corresponding responses to peripheral (humoral or electrical) stimulation (Fig. 5).

The behavioural changes induced by electrical stimulation of the posterior hypothalamus in unanaesthetized rats consisted of sudden arousal and fast exploratory activity culminating in a flight reaction. The enhanced locomotor activity usually subsided very soon after discontinuation of the stimulus, and in the periods between stimulation the rats did not show increased fear, in contrast to rats stimulated in other cerebral regions. Threshold intensity of the stimulus for a rise in blood pressure was somewhat lower than in anaesthetized rats. The degrees of inhibition of the pressor response to hypothalamic stimulation obtained with the four drugs under investigation were essentially the same as in anaesthetized rats. The

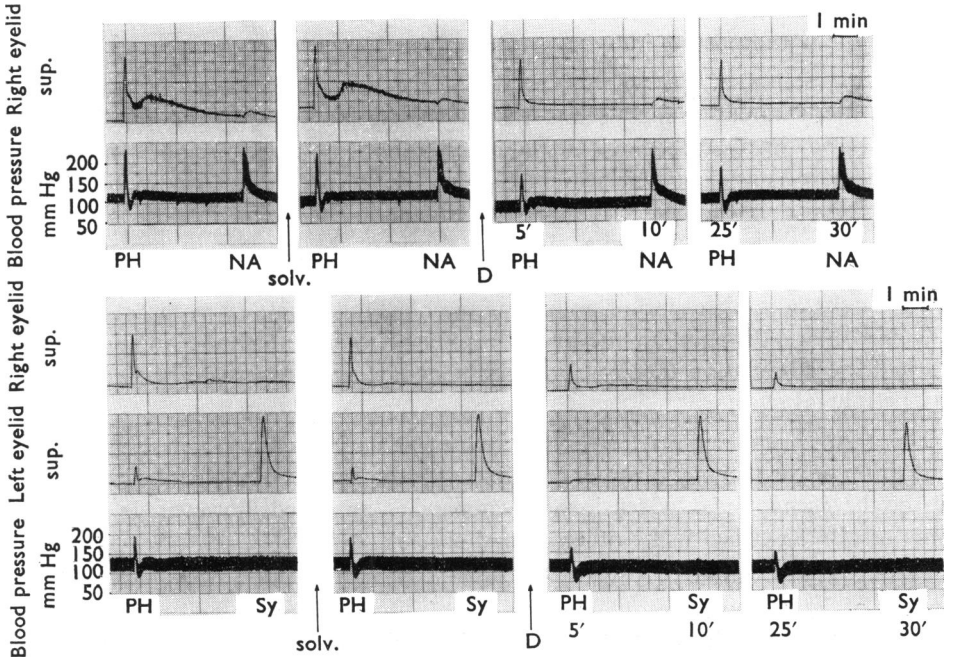

FIG. 5. Effects of diazepam on sympathetic responses in anaesthetized rats. At solv.: solvent* 1 ml./kg i.p.; at D: diazepam 5 mg/kg i.p. Upper tracings: PH=hypothalamic stimulation (4 V); NA=3 μg i.v. Lower tracings: PH=hypothalamic stimulation (11 V); Sy=stimulation of the left cervical sympathetic nerve (3 V). (The impairment of the eyelid responses to central stimulation on the side of the peripheral electrode can be noticed.)

* The solvent consisted of propylene glycol 40% ; ethanol 10% ; benzyl alcohol 1.5% ; sodium benzoate-benzoic acid 5% and water to 100%.

behavioural reaction was not affected by guanethidine and phentolamine. Chlorpromazine and diazepam, at a dose of 5 mg/kg intraperitoneally, did not abolish, but only delayed, the flight reaction, even when elicited with threshold intensity of stimulation. Basal locomotor activity was, however, greatly reduced both by chlorpromazine and by diazepam.

Discussion

The experiments reported in this paper indicate the possibility of characterizing substances affecting sympathetic functions by studying the way in which they modify pressor responses and eyelid contractions elicited by electrical stimulation of the posterior hypothalamus in anaesthetized rats.

Whereas several drugs have been investigated for their ability to modify the hypertensive response to eserine, which has been attributed to a central activation of the adrenergic neurones (Varagic, Krstic & Mihajlovic, 1964), the hypertensive response to direct stimulation of sympathetic centres in the rat has not been so extensively used in pharmacological studies. We found, however, that it can easily provide similar and probably more reliable results about drug effects. The changes in heart rate elicited by hypothalamic stimulation did not prove to be an adequate test for studying sympathetic functions. In our experiments the rise in blood pressure induced by hypothalamic stimulation in anaesthetized rats was never accompanied by tachycardia, in contrast to the results described by Folkow & Rubinstein (1966). Instead we observed a reduction in heart rate, as was always the case for the noradrenaline-induced pressor effect.

The contraction of the inferior eyelid of the anaesthetized rat in response to sympathetic stimulation has been studied by Gertner (1956) and employed by Spriggs (1966) in pharmacological studies on adrenergic transmission. In our preparation more consistent results were obtained by recording the contraction of the superior eyelid. Although a somatic component in the eyelid responses to hypothalamic stimulation cannot be excluded, the role of the autonomic nervous system was demonstrated by the inhibition obtained after section of the sympathetic nerve and after the administration of an adrenergic blocking agent. A comparison of the influence of a substance on the eyelid responses to hypothalamic and to cervical sympathetic stimulation therefore appears adequate to differentiate between central and peripheral effects.

The results obtained in the present experiments, which were designed for an evaluation of the technique, are compatible with the known pharmacological properties of the compounds. Occasional discrepancies appeared, however, in regard to some results described using other animal species.

Peripheral anti-adrenaline activity was demonstrated for phentolamine, for similar degrees of inhibition were obtained on the blood pressure rise and eyelid contractions in response to hypothalamic stimulation, and on the corresponding responses to noradrenaline and cervical sympathetic stimulation. A species difference could account for the different results obtained by Bergmann, Catane & Korczyn (1967), who suggested a central action of phentolamine on blood pressure.

A peripheral adrenergic neurone activity was demonstrated for guanethidine by the equal reduction of the eyelid responses to central and peripheral stimulation, and

by the blockade of the pressor response to hypothalamic stimulation in the absence of anti-adrenaline activity. The faster onset of inhibition of the pressor effect than the eyelid contractions also appears in the experiments of Spriggs (1966). It can be explained by a different rate of penetration or by a functional specificity of drug action on different adrenergic nerves, similar to observations made with bretylium on the responses to hypothalamic stimulation in anaesthetized cats (Morpurgo and Morillo, 1962) .

The results obtained with chlorpromazine depend on the organs involved and on the animal species tested. In the cat the noradrenaline-induced pressor response is not inhibited or may even be enhanced by chlorpromazine (Martin, Riehl & Unna, 1960) and inconsistent results have been found in the blood pressure rise induced by hypothalamic stimulation (Dasgupta & Werner, 1954 ; Schallek & Zabransky, 1966). In our experiments with anaesthetized rats the chlorpromazine-induced blockade of the pressor response to hypothalamic stimulation, which has also been reported by Schmitt (1966), paralleled a marked inhibition of the noradrenaline pressor effect. An adrenergic blocking activity of chlorpromazine has been demonstrated in the cat by the inhibition of the nictitating membrane contraction following cervical sympathetic stimulation (Thoenen, Hurlimann & Haefely, 1965), whereas it had been denied by Jourdan, Duchene-Marullaz & Boissier (1955) because it lacks activity against the responses to cervical sympathetic stimulation in dogs and rabbits. In the rat also we failed to obtain consistent inhibition of the eyelid response to stimulation of the superior cervical sympathetic nerve, in contrast to the inhibition of the response to hypothalamic stimulation. These results seem to indicate, in agreement with the evidence presented by Tedeschi (1967), a decrease of the central sympathetic outflow induced by chlorpromazine.

For diazepam a central site of action is suggested by the reduction of sympathetic responses to hypothalamic stimulation without a significant effect on the corresponding peripherally evoked responses. This is in agreement with the results obtained by Schallek & Zabransky (1966) in unanaesthetized cats. Diazepam, however, appeared much less potent in inhibiting the blood pressure rise induced by hypothalamic stimulation in the rat.

The measurement of pressor responses to hypothalamic stimulation in conscious rats has allowed the simultaneous observation of behavioural reactions and the recovery from drugs without the interference of an anaesthetic. For the assessment of drug effects on the autonomic system, however, the results were not more informative than those obtained in urethane-treated rats.

The flight reaction induced by electrical stimulation of the posterior hypothalamus appeared to be highly resistant to the inhibitory activity of drugs and independent to peripheral sympathetic responses. A strong flight reaction was observed when the pressor rise was blocked by the administration of phentolamine or guanethidine, and, conversely, a marked increase of blood pressure, as effected by an intravenous noradrenaline injection, did not change the behaviour of conscious rats (unpublished). Even with CNS-active drugs the behavioural reaction to hypothalamic stimulation was scarcely inhibited at doses already impairing spontaneous motor activity.

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