

EXPERIMENTAL STUDIES ON HEADACHE: PHARMACODYNAMICS OF URINE EXCRETED DURING MIGRAINE HEADACHE AND ITS RELATION TO 17-KETOSTEROID CONTENT

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In the search for a pharmacodynamically active substance, excreted in the urine during an attack of migraine headache, the contraction producing effect of urine on the *rectus abdominis* muscle of the frog was investigated.

EXPERIMENTAL PROCEDURES

In preliminary experiments, 292 specimens of urine from 18 healthy, non-migrainous adults (8 males and 10 females) were assayed. Samples collected either at the same hour for several days, or at random intervals during consecutive days, were separately assayed.

The urine was tested immediately after excretion or was acidified by the addition of approximately 3 cc. of concentrated HCl to 100 cc. urine and kept in a refrigerator for later use. Before use, the pH was corrected to 7 with an 8 molar NaOH or HCl solution. The *rectus abdominis* muscle was immersed in a 20 cc. muscle chamber containing, alternately, Ringer's solution for 10 minutes, followed by a 20 mM potassium chloride solution for 30 seconds. The height of contraction was registered by an isotonic lever on a kymograph. After the height of muscle contraction induced by potassium ions remained constant for 3 successive immersions, the muscle was placed for 30 seconds in the urine to be tested.

It was found that the urine of healthy adults induces contraction of the *rectus abdominis* muscle of the frog. Male urine is on the average 70 per cent more effective in inducing contraction than is female urine. The magnitude of contraction caused by urine varies from person to person but remains fairly constant for one person over a period of a few days. In the women studied, the urine excreted during the premenstrual period induced greater contraction of the muscle than did urine excreted subsequent to the menstrual period, the average increase in the 8 instances investigated being 50 per cent.

The effect on the *rectus abdominis* muscle of 379 specimens of urine, collected from 12 migraine patients (6 males and 6 females) during attack-free periods and during the different phases of the attack of migraine headache, was studied. It was found that urine collected during the prodromic period of a migraine headache attack caused the muscle to contract somewhat less than did urine collected before or the day after the attack, while specimens collected during the headache period induced a greater contraction of the muscle (Table I, Figure 1).

The specific gravity of the urine excreted during the headache period of the migraine attack was low, except in a few instances. All samples, regardless of variations in specific gravity, induced a much greater contraction of the *rectus abdominis* muscle than did samples collected in attack-free periods.

Attempts were made to identify the substance responsible for this phenomenon. It was found that samples which induced a greater muscle contraction contained more 17-ketosteroid compounds.¹

1. Chemical determinations

The ketosteroid content of 100 cc. samples was determined by the method of Zimmerman as modified by Callow (1). Urine collected during headache periods contained more 17-ketosteroid compounds than specimens collected during attack-free periods (Table II, Figure 1). Since the latter method is a colorimetric one, errors may be introduced by the presence of other substances in

TABLE I
Contraction-producing effect of urine collected during an attack of migraine headache

Time of collection	Number of experiments	Changes of the height of contraction in percentage of control (The control is 100 per cent)	
		Mean	S.E. of mean*
Day before attack	40	0	
Prodromic period	8	- 40	± 4.2
Headache	44	+ 194	± 17.1
Day after attack	35	+ 5	± 0.7

* S.E. of mean $\sqrt{\frac{\Sigma(\Delta)^2}{N(N-1)}}$ Σ = summation
 Δ = deviation of each sample from the mean
 N = number of experiments

¹ It must be emphasized that although the magnitude of contraction of the *rectus abdominis* muscle, as assayed in these experiments, increased with the concentration of a chemical agent, this increase did not parallel the increase in the concentration of the agent. Thus, with a linear increase in the concentration of an active substance, the graphic representation of the increase in the height of contraction ascends more abruptly.

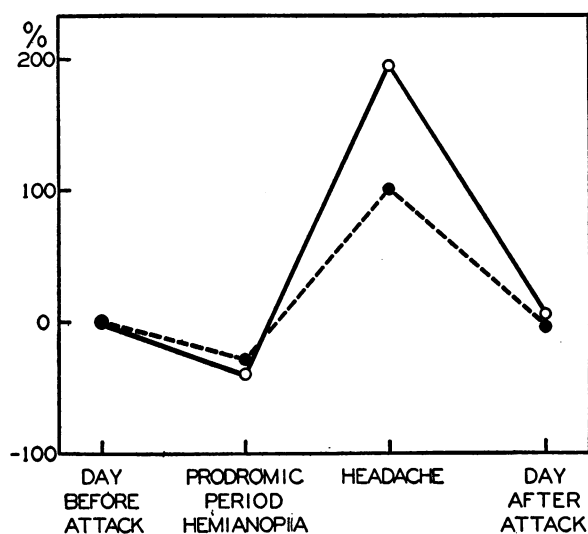


FIG. 1. COMPARISON OF THE 17-KETOSTEROID CONTENT AND THE CONTRACTION-PRODUCING EFFECT OF URINE

○—○ Changes of the height of contraction in percentage of control.

●-----● Changes of the 17-ketosteroid content in percentage of control.

the urine, giving a similar color reaction. Therefore, the actual 17-ketosteroid content of the urine may be lower than the values given in Table II, but the magnitude of the changes is indicative of an actual increase of the 17-ketosteroid content of the urine.

2. Biological observations

I. Healthy male and female subjects were injected intramuscularly with 25 mgm. testosterone propionate (Oreton, Schering). The urine was collected before injection, and 4, and 24 hours after injection. Samples collected after injection caused the *rectus abdominis* muscle to contract more than those collected before (Table IV). Injected testosterone is partly eliminated in the urine as 17-ketosteroid compounds (2 to 5).

II. The urine of an acromegalic male could not be demonstrated to contain 17-ketosteroid compounds. Samples of this urine induced 80 per cent less muscle contraction than did the urine of healthy male adults.

III. The urine of 5 children below 3 years of age also induced 80 per cent less contraction of the *rectus abdominis* muscle than did the urine of healthy adult males. It is known that children

below 3 years of age eliminate only minimal amounts of 17-ketosteroid compounds (6).

IV. Samples collected during the premenstrual days (8 instances), with an increased contracting effect, contained more 17-ketosteroid compounds.

The inference that the active agent in the urine of migraine attacks was identical with one of the 17-ketosteroid compounds would be strengthened if an amount of the 17-ketosteroid compound equivalent to that in the urine reproduced, when added to normal urine, similar effects on the *rectus abdominis* muscle. Since the compound is not yet isolated, 17-ketosteroid compounds actually known to be eliminated in the urine and other steroid compounds which do not appear in the urine were added to samples of normal urine. It was found that these steroid compounds potentiate the contraction-producing effect of normal urine to an extent comparable to that of the headache urine (Table III). An evaluation of the smallest amount having a potentiating effect, and of the correlation between concentration and contraction-producing power of the steroid compounds is difficult, because the slight water solubility of the substances prevented the estimation of the amount dissolved. Furthermore, the concentrations could not be increased at will. It may be assumed, however, that concentrations existing in the body fluids could potentiate the contraction of striated muscle (7).

These observations suggest that some of the derivatives of the steroid hormones may be responsible for the increased effect of the migraine urine on striated muscle.

Thus far, the argument has been based on the

TABLE II
The 17-ketosteroid content of urine collected during an attack of migraine headache

Time of collection	17-ketosteroid content				Changes of the 17-ketosteroid content. Mean of 19 attacks
	Male (9 attacks)		Female (10 attacks)		
	Mean	S.E.	Mean	S.E.	
	mgm. per 100 cc.				per cent of control
Day before attack	1.22	±0.102	0.96	±0.077	0
Prodromic period	0.82	±0.061	0.60	±0.049	- 35
Headache	2.42	±0.113	1.91	±0.120	+ 100
Day after attack	1.15	±0.095	0.91	±0.082	- 4

TABLE III
Effect of steroid compounds on the contraction-producing effect of normal urine

Substance added to urine	Concentration in 100 cc.	Number of experiments	Increase of the height of contraction	
			Mean	S.E.
			<i>per cent of control</i>	
Dehydroisoandrosterone (Schering).....	Traces	8	30	±3.0
Androsterone (Ayerst, McKenna, Harrison).....	Traces	10	37	±3.5
Acetate of α -hydroxy-etiocholanone-17 (Ayerst, McKenna, Harrison).....	Traces	10	27	±2.9
Sodium pregnandiol glucuronidate (Ciba).....	1 mgm.	10	40	±3.2
Testosterone propionate (Ciba).....	Traces	8	53	±5.0
Testosterone propionate (Schering).....	Traces	8	41	±3.1
Progesterone (Roche-Organon).....	Traces	8	35	±4.0
Progesterone (Ciba).....	Traces	8	42	±2.9
Cholesterol.....	0.1 mgm.	10	34	±3.0

assumption that the pharmacodynamic action of the urine is based on the presence of an additional factor during migraine headache attack. May the effect of the urine of migraine patients be due to the lack of a substance which is present in normal urine and which depresses the contraction-producing effect of normal urine? If such an assumption be considered, one asks at once what substances present in the normal urine and absent in the urine of migraine attacks could act as depressor agents. Since migraine headache attacks are commonly associated with diuresis, it is conceivable that an antidiuretic factor exists in the urine of healthy subjects, which could exert such a depressor effect. Therefore, attempts have been made to determine whether the antidiuretic factor could exert a depressor effect on the *rectus abdominis* muscle. The muscle was immersed in a solution of the antidiuretic principle of the posterior pituitary gland (Pitressin, Parke, Davis & Co.). It was discovered that rather than having a depressor effect on the *rectus abdominis* muscle, the antidiuretic principle (50 I.U. per 100 cc.), increased on the average by 24 per cent (S.E. = ± 3.5) the effect of urine in producing a contraction (24 experiments). It may therefore be inferred that the increase of the contracting effect of the urine collected during the migraine headache cannot be explained by the absence of the antidiuretic component of the pituitary gland.

Since it is conceivable that Pitressin may contain traces of other pituitary hormones, such as the oxytocic principle, the effect on the *rectus abdominis* muscle of the oxytocic factor of the posterior pituitary gland (Pitocin, Parke, Davis & Co.) was

also investigated. Pitocin, when added to urine even in large amounts (100 I.U. per 100 cc.), did not potentiate the muscle contraction.

The degree to which other substances modify the contraction-producing effect of urine was investigated as described below.

Potassium. The potassium content of the samples excreted during an attack of migraine headache was determined by the biological method of Torda (8). Potassium is a potent agent in producing muscle contraction, but calcium in higher concentrations almost completely counteracts this effect. Calcium chloride was therefore added to the urine until no further precipitation occurred. This end-point was determined by repeated centrifugation. Then calcium chloride was added in a concentration of 54 mM. The *rectus abdominis* muscle was immersed in the original urine for 30 seconds, washed with Ringer's solution for 10 minutes, and immersed in the calcium-urine for 30 seconds. Samples of normal urine, containing a larger amount of added potassium than the migraineous urine could contain, lost, on the average, 79 per cent (S.E. = ± 1.2) of their contraction-producing effect after the addition of calcium, while the urine collected during the migraine headache lost only 16 per cent (S.E. = ± 1.5) of its contraction-producing effect. This indicates that the contraction produced by the original urine was mainly due to another substance and not to potassium.

Acetylcholine. The contraction caused by urine collected during an attack of migraine headache was not due to acetylcholine because

1. The contraction-producing agent excreted in

TABLE IV
Effect of injected testosterone and histamine on the contraction-producing effect of urine
(healthy subjects without an attack of migraine)

Substance	Route of administration	Amount injected	Number of experiments	Increase of the height of contraction				
				Urine collected				
				Before injection	4 hours later		24 hours later	
					Mean	S.E.	Mean	S.E.
Testosterone propionate	Intramuscular	25 mgm.	6	0	<i>per cent of control</i>		42	±3.5
Histamine	Intravenous	1 mgm.	8	0	250	±20.3	1	±0.7
					3	± 2.0		

the urine maintained its activity unchanged for several days;

2. The amount of contraction of the *rectus abdominis* muscle did not change if the muscle was immersed in a 0.4 mgm. per 100 cc. physostigmine salycilate solution, for 20 minutes between 2 immersions in the urine. The acetylcholine sensitivity of the *rectus abdominis* muscle for a similar treatment increases from 200 to 300 per cent (18 experiments);

3. The magnitude of contraction of the *rectus abdominis* muscle did not change if the muscle was immersed in a 6 mgm. per 100 cc. atropine sulfate solution, for 30 minutes between 2 immersions in the urine. Were these effects on the *rectus abdominis* muscle due to acetylcholine, the aforementioned concentration of atropine would completely abolish the muscle contraction (9). Atropine in the concentration used does not modify the sensitivity of the striated muscle to potassium stimulation (9) (12 experiments);

4. Boiling did not decrease but usually increased the contraction-producing effect of urine (18 experiments).

Histamine

1. Histamine phosphate was injected, in healthy males and females, in concentrations large enough to produce headache (1 to 1.5 mgm. subcutaneously or 0.1 mgm. intravenously). Urine collected hourly for 6 hours after injection did not induce a greater contraction of the *rectus abdominis* muscle than did specimens collected before the injection (Table IV).

2. Histamine may produce a slight increase (16

per cent) of the contraction-inducing effect of urine. The optimal concentration used in this experimental procedure was 0.5 mgm. per cent.

3. Chemical determinations (method of Koessler and Hanke, (10)) did not reveal histamine in the urine. To be sure this chemical method does not detect minimal quantities of histamine, but it does detect smaller concentrations than are shown to be necessary to potentiate muscle contraction.

DISCUSSION

Urine collected during an attack of migraine headache caused the *rectus abdominis* muscle of the frog to contract to a greater degree than did specimens collected during the attack-free periods. The 17-ketosteroid content of the urine increased with the contraction-producing effect, as demonstrated by chemical determinations and biological experiments. Specimens collected during the prodromic period induced the least contraction and contained less 17-ketosteroid compounds. The slight decrease of the 17-ketosteroid content and contraction-producing effect of urine collected during the prodromic period may be due to decreased excretion by the kidney, resulting from concurrent generalized vasoconstriction (11).

The relation of the 17-ketosteroids, or the substances from which they arise, to the attack of migraine headache cannot yet be formulated. It is possible that the increased elimination of the 17-ketosteroid compounds has no direct bearing on the headache mechanism and is merely an epiphenomenon such as is, for example, diarrhea. It may represent a secondary hypersecretion of the steroid hormones, or conceivably a decreased retention of

steroid compounds in the blood or tissues; it may even be a manifestation of modified metabolism of sterols in the liver.

There is evidence that, in subjects with migraine, the attack of headache comes during or immediately after a period of sustained tension, exhaustion, frustration, or emotional conflict (12). The increase of elimination of 17-ketosteroid compounds during the attack of headache is probably related to the severe stress which precedes or accompanies the attack. Indeed, it has been shown that stress alone may induce moderate increase in the 17-ketosteroid output (13, 14). It is not a result of the severe pain *per se*, experienced during the headache period of the migraine attack, because pain alone (sinusitis, childbirth, etc.) may be associated with a lowered elimination of 17-ketosteroids (14).

It has been shown that cranial vasodilatation is responsible for the headache during a migraine attack (15, 16). Also, it is known that most of the steroid hormones cause dilatation of the skin capillaries (17). Steroid compounds, if injected, seldom produce headache, but an increase in the steroid content of the body may play a part in the production of headache through potentiation of the effect of a locally liberated neurohumoral vasodilator substance such as acetylcholine.

SUMMARY AND CONCLUSIONS

1. Urine collected during attacks of migraine headache caused the *rectus abdominis* muscle of the frog to contract to a greater degree than did specimens collected during the attack-free periods. Specimens collected during the prodromic period induced least contraction.

2. The 17-ketosteroid content of urine increased with an increase in the contraction-producing effect, as demonstrated by chemical and biological assays.

3. The increase in the amount of contraction produced by urine collected during an attack of migraine headache was probably not due to potassium, acetylcholine, or histamine, nor was it related to the specific gravity of the urine.

4. It is suggested that steroid compounds may participate in the production of an attack of migraine headache by potentiation of the effect of a

locally liberated vasodilator neurohumoral substance.

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