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SHORT COMMUNICATION

The influence of caffeine and theobromine on locomotive activity and the brain cGMP/cAMP ratio in white mice

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The cyclic nucleotides adenosine 3,5 monophosphate (cAMP) and guanosine 3,5 monophosphate (cGMP) have been implicated as intracerebral target systems for the effects of centrally depolarizing drugs [1, 2] and also for the stress condition [3]. Electrophysiological studies by Stone *et al.* [4] and Phillis [5] have shown that cyclic nucleotides administered iontophoretically exert marked effect on the neuronal firing pattern of pyramidal tract neurons: cGMP had a predominantly excitatory action while spontaneously active neurons were depressed by cAMP.

It is well established that caffeine increases locomotive activity in white mice [6–10]. This could be explained as an activation of the pyramidal tract neurons resulting in an elevated discharge rate. It is not clear whether the cyclic nucleotides are involved in this effect: Some authors found a decrease in the cAMP content in certain areas of the brain after administration of caffeine or theophylline [11, 12] which was denied by others [13–15]. An increase of the cAMP level was also reported [12]. The influence of caffeine on the brain concentration of cGMP has not been investigated so far. We have recently shown that the caffeine-induced increase in locomotive activity and oxygen consumption in white mice was antagonized by theobromine [10]. The aim of the present study was (i) to establish the correlation between the caffeine-induced locomotive activity and the cGMP/cAMP ratio in the brain, (ii) to assess the effects of theobromine on these parameters and (iii) to investigate whether a theobromine pretreatment might alter the effect of caffeine on the cGMP/cAMP ratio.

From the experiments by Stone and Phillis [4, 5] showing an adverse effect of cGMP and cAMP on the firing pattern of pyramidal neurons, it seems that this ratio cGMP/cAMP might be more important for the electrophysiological activity of the brain cell rather than the actual amount of cGMP and cAMP present.

MATERIALS AND METHODS

Male NMRI mice (body weight 25 ± 1 g) which had free access to standard diet (FUKO^R) and tap water were used. The test substances (solubilizer: sodiumsalizylate) 45 μg/g caffeine, 180 μg/g sodium theobromine and 45 μg/g caffeine together with 180 μg/g sodium theobromine were dissolved in saline (0.9%) and injected intraperitoneally in a vol. of 0.25 ml. Control animals were injected with saline (0.9%).

The locomotive activity was determined in groups of five mice, 0–30 min after i.p. injection of the test substances with the aid of an Animex DO activity meter (Farad electronics, Sweden). All experiments were performed at the same time of day.

The determination of cyclic AMP and cyclic GMP was carried out using a protein binding method [16] modified for cAMP as initially described by Brown *et al.* [17] and for cGMP as described by Illiano *et al.* [18]. 30 min after i.p. injection of the test substances the mice were killed by microwave irradiation. The brains were dissected and rapidly frozen in liquid nitrogen. Tissues were homogenized (Potter S, Braun, Melsungen, W. Germany) in 5% trichloracetic acid. After centrifugation, the supernatant was pipetted off and washed five times with 10 vol. of water-saturated diethyl-ether. Residual ether was removed in a steam of nitrogen. For cyclic AMP assay, portions of the washed extract were lyophilized and subsequently taken up in distilled water. In the cyclic GMP assay, chromatography on Dowex 50 WX 8, 100–200 mesh, H^+ Form (Serva, Heidelberg) was used for further purification. The final cGMP fraction was collected, lyophilized and subsequently taken up in distilled water. To calculate losses of cGMP during the preparation, tracer quantities [^{3}H]-cGMP were added to the samples. Aliquots of the trichloracetic acid homogenates were assayed for protein according to Lowry *et al.* [19].

Statistical analysis was performed using student's *t*-test and differences were regarded to be significant if p was 0.05 or less.

RESULTS

Effects of caffeine and theobromine on the locomotive activity. As shown in Fig. 1a, 45 μg/g caffeine initiates a 90 per cent increase in the locomotive activity of white mice. This effect was completely antagonized by 180 μg/g theobromine. Theobromine alone had no influence on the motor activity.

Effects of caffeine and theobromine on the brain concentration of cyclic nucleotides. Table 1 shows the total brain concentration of cAMP which was diminished by 31% 30 min after caffeine. Theobromine had no effect on the brain cAMP when given by itself but completely prevented the caffeine induced decrease. By contrast, total brain cGMP increased by 49% 30 min. after caffeine. Again this effect was prevented by theobromine-pretreatment which was ineffective when given alone.

Fig. 1b shows the cGMP/cAMP ratio in these experiments. It was elevated after caffeine but remained unchanged after theobromine or caffeine and theobromine, respectively. Thus a similar graphical pattern as in the locomotion experiments was obtained.

DISCUSSION

Our experiments have shown that the increase in locomotive activity of white mice after caffeine was paralleled by a rise in the cGMP/cAMP ratio in the brain. Theobromine, though ineffective by itself, completely prevented these effects. As caffeine and theobromine are structurally similar, one might assume that a competitive antagonism exists between these methylxanthines at a certain brain receptor.

The findings by Stone *et al.* [4] and Phillis [5] showing an increase in electrical activity by cGMP and a decrease by cAMP in pyramidal neurons of the rat and cat brain, and thus a counteraction of cGMP and cAMP, are in agreement with our results. It may be concluded that caffeine raises cGMP by activating the guanylate cyclase and at the same time diminishes cAMP by inactivating the

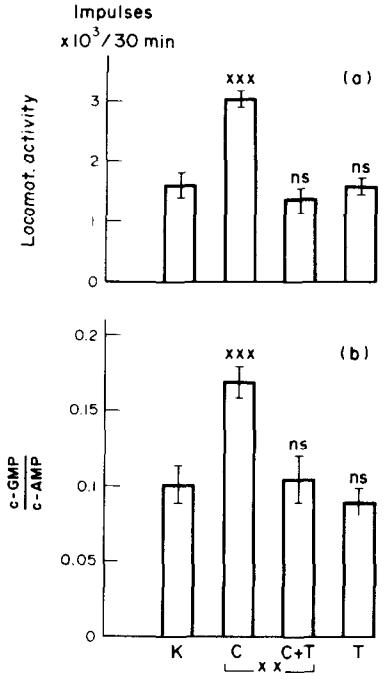

Fig. 1. Male white mice, body weight 25 ± 1 g, shown are means ± S.E.M. ($n \geqq 5$). (a) Motor. activity: impulses × 10^3 0–30 min after 0.9% NaCl (K), 45 μg/g caffeine (C), 180 μg/g theobromine (T), 45 μg/g caffeine + 180 μg/g theobromine (C + T). (b) Brain ratio cGMP/cAMP 30 min after 0.9% NaCl (K), 45 μg/g caffeine (C), 180 μg/g theobromine (T), and 45 μg/g caffeine + 180 μg/g theobromine (C + T). Total brain concentrations of controls: cAMP 9.65 ± 0.36 pmoles/mg protein, cGMP 1.0 ± 0.12 pmoles/mg protein. xxx $P \leqq 0.001$, xx $P \leqq 0.01$, n.s. = no significance to controls.

adenylate cyclase resulting in an increase of the cGMP/cAMP ratio.

It is unlikely that the effect of caffeine on the cGMP/cAMP ratio was due to its ability to inhibit the phosphodiesterase; for in that case one would expect an increase in the cAMP level as well. On the other hand it has been shown by Vernikos-Dannellis and Harris [20] that caffeine and theophylline had no influence on the rat brain phosphodiesterase. Furthermore, it is not yet clear whether phosphodiesterases play any functional role in the brain [21, 22].

Table 1. Total brain concentration of c-AMP and c-GMP after administration of saline, caffeine, theobromine and caffeine + theobromine*

Substrate	c-AMP	c-GMP
Saline (0.9%)	9.65 ± 0.36	1.00 ± 0.12
Caffeine (45 μg/g)	6.62 ± 0.87	1.49 ± 0.30
Caffeine (45 μg/g) + theobromine (180 μg/g)	9.80 ± 0.65	1.03 ± 0.12
Theobromine (180 μg/g)	8.50 ± 1.09	0.79 ± 0.16

* Values are expressed as means in pmoles/mg protein ± S.E.M.

One might speculate from our findings that the caffeine induced increase in locomotive activity is causally related to the elevation in the cGMP/cAMP ratio. Further work will clearly be needed to obtain conclusive data.

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REFERENCES

1. C. C. Mao, A. Guidotti and E. Costa, *Naunyn-Schmiedeberg's Arch. exp. Path. Pharmac.* **289**, 369 (1975).
2. F. A. Opmeer, S. W. Gumulka, V. Dinnendahl and P. S. Schönhöfer, *Nauyn-Schmiedeberg's Arch. exp. Path. Pharmac.* **292**, 259 (1976).
3. V. Dinnendahl, *Brain Res.* **100**, 716 (1975).
4. T. W. Stone, D. A. Taylor and F. E. Bloom, *Science, N.Y.* **187**, 845 (1975).
5. J. W. Phillis, *Adv. Behav. Biol.* **10**, 57 (1974).
6. A. Herz, B. Neteler and H. J. Teschemacher, *Arch. exp. Path. Pharmak.* **261**, 486 (1968).
7. J. R. Boissier and P. Simon, *Archs int. Pharmacodyn.* **158**, 212 (1965).
8. J. R. Boissier and P. Simon, *Archs int. Pharmacodyn.* **166**, 362 (1967).
9. F. Heim and B. Haas, *Arch. exp. Path. Pharmak.* **226**, 395 (1955).
10. F. Heim, B. Hach, P. Mitznegg, H. P. T. Ammon and C.-J. Estler, *Arzneimittel.-Forsch.* **21**, 1039 (1971).
11. J. K. Palmer, *J. Agr. Food. Chem.* **21**, 923 (1973).
12. M. I. Paul, G. L. Pauk and B. R. Ditzion, *Pharmacology* **3**, 148 (1970).
13. S. Kakiuchi, T. W. Rall and H. McIlwain, *J. Neurochem.* **16**, 485 (1969).
14. J. Forn and G. Krishna, *Pharmacology* **5**, 193 (1971).
15. A. Sattin, *J. Neurochem.* **18**, 1087 (1971).
16. A. G. Gilmann, *Proc. natn. Acad. Sci. U.S.A.* **67**, 305 (1970).
17. B. L. Brown, J. D. M. Albano, R. P. Ekins and A. M. Sgherzi, *Biochem. J.* **121**, 561 (1971).
18. G. Illiano, G. P. E. Tell, M. I. Siegel and P. Cuatrecasas, *Proc. natn. Acad. Sci. U.S.A.* **70**, 2443 (1975).
19. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
20. J. Vernikos-Dannellis and C. G. Harris, *Proc. Soc. exp. Biol. Med.* **128**, 1016 (1968).
21. T. W. Rall, A. Sattin, in *Advances in Biochemical Psychopharmacology* (Eds. P. Greengard and E. Costa) Vol. 3, pp. 113–133. Raven Press, New York (1970).
22. A. Sattin, T. W. Rall, *Molec. Pharmac.* **6**, 13 (1970).