REFERENCES

ALLEE, W. C. (1942). *Science*, **95**, 289.

ANDÉN, N.-E., CORRODI, H., FUXE, K., HÖKFELT, B., RYDIN, C. & SVENSSON, T. (1970). *Life Sci.*, **7**, 513–523.

ANTON, A. H. & SAYRE, D. F. (1962). *J. Pharmac. exp. Ther.*, **138**, 360–374.

CARLSSON, P. A. E., CORRODI, H. R., FLORVALL, G. L. & ROSS, S. B. (1970). Austrian patent No. 284 143.

CREVELING, C. R., DALY, J., TOKUYMA, T. & WITKOP, B. (1968). *Biochem. Pharmac.*, **17**, 65–70.

ESSMAN, W. B. (1969). In: *Aggressive behaviour*. pp. 233–238, Editors: Garattini, S. and Sigg, E. B., Amsterdam: Excerpta Medica Foundation.

EVERETT, G. M., DAVIS, J. C. & TOMAN, J. E. (1959). *Fedn Proc. Fedn Am. Socs exp. Biol.*, **18**, 388.

FLORVALL, L. & CORRODI, H. (1970). *Acta pharm. suecica*, **7**, 7–22.

GARATTINI, S., GIACALONE, E. & VALZELLI, L. (1969). In: *Aggressive behaviour*, pp. 179–187, Editors: Garattini, S. and Sigg, E. B., Amsterdam: Excerpta Medica Foundation.

GOLDBERG, M. E., DUBNICK, B., HEFNER, M. & SALAMA, A. I. (1973a). *Neuropharmac.*, **12**, 249–260.

GOLDBERG, M. E., INSALACO, J. R., HEFNER, M. A. & SALAMA, A. I. (1973b). *Ibid.* **12**, 1049–1058.

GOODWIN, B. L., JOHNSON, R. D., LEASK, B. G. S., RUTHVEN, C. R. J. & SANDLER, M. (1972). *Experientia* (Basel), **28**, 1298–1299.

HODGE, G. K. & BUTCHER, L. L. (1975). *Eur. J. Pharmac.*, **31**, 81–93.

MORPURGO, C. (1968). *Ibid.*, **3**, 374–377.

JANSSEN, P. A. J., JAGENAU, A. H. & NIEMEGEERS, C. J. E. (1960). *J. Pharmac. exp. Ther.*, **129**, 471–475.

ROSS, S. B., RENYI, A. L. & ÖGREN, S.-O. (1972). *Eur. J. Pharmac.*, **17**, 107–112.

SCHMITT, H., SCHMITT, H. & FENARD, S. (1971). *Ibid.*, **14**, 98–100.

SCOTT, J. P. (1946). *J. comp. Psychol.*, **39**, 379–390.

STARKE, K., MONTEL, H., GAYK, W. & MERKER, R. (1974). *Naunyn-Smiedebergs Arch. Pharmac.*, **285**, 133–150

VALZELLI, L. L., GIACALONE, E. & GARATTINI, (1967). *Eur. J. Pharmac.*, **2**, 144–146.

WELCH, B. L. & WELCH, A. S. (1969). In: *Aggressive behaviour*, pp. 188–292, Editors: Garattini, S. & Sigg, E. B., Amsterdam: Excerpta Medica Foundation.

YEN, C. Y., STANGER, R. L. & MILLMAN, N. (1959). *Archs int. Pharmacodyn. Thér.*, **123**, 179–185.

Apomorphine as an antagonist of the dopamine response from the nucleus accumbens

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The nucleus accumbens has been subjected to extensive investigation as a site at which dopamine and dopamine agonists are able to initiate hyperactivity (Pijnenburg & van Rossum, 1973; Elkhawad & Woodruff, 1975; Kelly, Seviour & Iversen, 1975; Pijnenburg, Honig & van Rossum, 1975; Costall & Naylor, 1975, 1976; Costall, Naylor & Pinder, 1976; Pijnenburg, Honig & others, 1976) and a number of models, based on the effect of dopamine in this area, have been proposed for the detection of both dopamine agonist (Iversen, Kelly & others, 1975; Kelly, 1975; Kelly, Miller & Neumeyer, 1975) and antagonist activity (Costall & Naylor, 1976). We have been particularly interested in a model proposed by Iversen and her colleagues in which 6-hydroxydopamine is injected into the nucleus accumbens to increase the sensitivity of the dopamine receptors in this area and render an animal more sensitive to the hyperactivity inducing effect of dopamine agonists. However, we find one major difficulty in interpretation of data from this model: following 6-hydroxydopamine injections into the nucleus accumbens apomorphine is shown to induce a marked locomotor response (Iversen & others, 1975) yet we find that apomorphine is not a stimulant of locomotor activity in normal rats and does not induce a hyperactivity when injected directly into the nucleus accumbens of normal animals (Costall, Naylor & Neumeyer, 1975a). Further, in our hands, injections of 6-hydroxydopamine into the nucleus accumbens (which fail to significantly modify dopamine content of the tuberculum olfactorium) fail to render animals sensitive to a hyperactivity component of the apomorphine effect, either when apomorphine is injected by a peripheral route or directly into the 6-hydroxydopamine-treated nucleus accumbens. However, if 6-hydroxydopamine is placed in the tuberculum olfactorium, apomorphine may then produce a hyperactivity (Costall & others, in preparation). This would tend to emphasise a point which has been raised by Iversen and her colleagues that the response to apomorphine they observed may

* Correspondence

be dependent on changes in the tuberculum olfactorium which was affected by their 6-hydroxydopamine lesions. To further analyse the possible changes in dopamine activity which may be caused by apomorphine in the nucleus accumbens, we have investigated the changes which apomorphine and other dopamine agonists may cause in the hyperactivity response following injections of dopamine itself into the nucleus accumbens. When hyperactivity was established to injections of dopamine, apomorphine and other dopamine agonists, (−)-*N*-n-propylnorapomorphine (−)-NPA, bromocriptine, amphetamine and piribedil, were administered either peripherally or directly into the nucleus accumbens and any changes in the dopamine response determined.

Male Sprague-Dawley rats were used and stainless steel guide cannulae for bilateral injections into the nucleus accumbens were stereotaxically implanted in their brains (Costall & Naylor, 1975). The locations of these cannulae were checked histologically and all locations were correct for injections into the area of the nucleus accumbens as previously reported (Costall & Naylor, 1975, 1976). Animals were first used in the hyperactivity studies 10–14 days after surgery. Stainless-steel stylets, which had kept the guides patent, were replaced by bilateral, stainless-steel injection units which terminated 2·5 mm below the tips of the guides at the centre of the nucleus accumbens (Ant. 9·0, Vert. 0, Lat. ±1·6; De Groot, 1959). All animals received nialamide (100 mg kg^{-1}, i.p.) 2 h before dopamine. To determine any possible antagonistic effects of the dopamine agonists on the dopamine response, the latter was administered in a dose of 50 μg in 1 μl from micrometer syringes attached to the injection units (see Costall & Naylor, 1976 for details). Previous studies have shown that 50 μg dopamine induces a maximum hyperactivity response. A lower dose of dopamine, 6·25 μg μl^{-1}, was used to determine any possible enhancement of the dopamine response by the dopamine agonists. Immediately after an intracerebral injection, rats were placed in individual Perspex activity boxes fitted with photocells. Each interruption of the light beam was recorded and the total noted every 10 min. Activity was then expressed in counts per 5 min. The activity boxes were located in a sound-proofed room, diffusely illuminated and maintained at 21 ± 1°.

The hyperactivity induced by dopamine was a maximum 2·5 h after its injection into the nucleus accumbens. Animals were then given apomorphine and (−)-NPA subcutaneously or bromocriptine, piribedil and amphetamine intraperitoneally (apomorphine HCl, Macfarlan Smith, and (−)-NPA HCl, Neumeyer, were prepared in distilled water containing 0·1% sodium metabisulphite, bromocriptine methanesulphonate, Sandoz, in a minimum quantity of tartaric acid made up to volume with distilled water, and (+)-amphetamine SO_4, Sigma, and piribedil monomethanesulphonate, Servier, were dissolved in distilled water) and activity was recorded for a further 4·5 h. Sub-stereotypic doses of apomorphine (0·0625–0·25 mg kg^{-1}, s.c.) caused dose-dependent reductions in the dopamine-induced hyperactivity ($P<0·001$ at all doses) (Fig. 1). This inhibition occurred within 10 min and persisted for up to 90 min. (−)-NPA also reduced the hyperactivity response in a dose-dependent manner ($P<0·01$–$P<0·001$) (Fig. 1) but the doses required were within the range which also induce periodic biting (Costall, Naylor & Neumeyer, 1975b), although

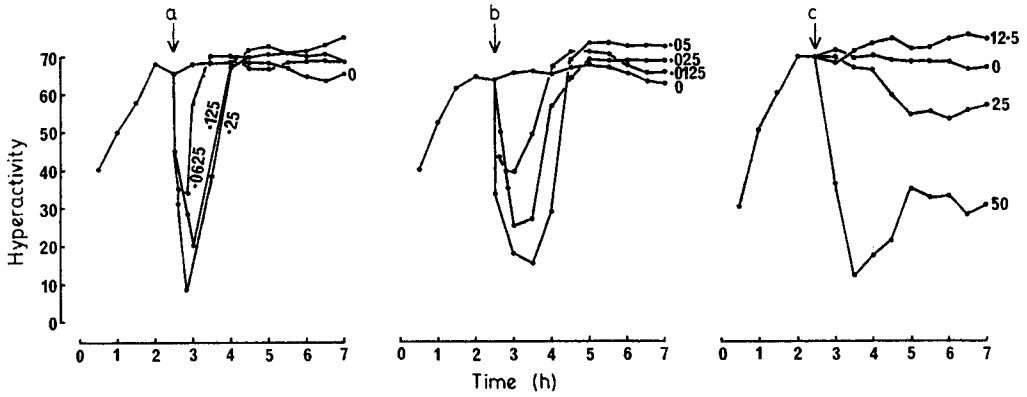

FIG. 1. Antagonism by a-apomorphine, b-(—)-NPA and c-bromocriptine of the hyperactivity induced by 50 μg μl^{-1} dopamine administered into the nucleus accumbens 2 h after pretreatment with nialamide (100 mg kg^{-1}, i.p.). Apomorphine and (—)-NPA were administered subcutaneously and bromocriptine by the intraperitoneal route 2·5 h after dopamine (↓). Hyperactivity is expressed in counts per 5 min. 6–10 rats were used at each dose level of dopamine agonist which is indicated in mg kg^{-1}. Standard errors on the means are less than 14%.

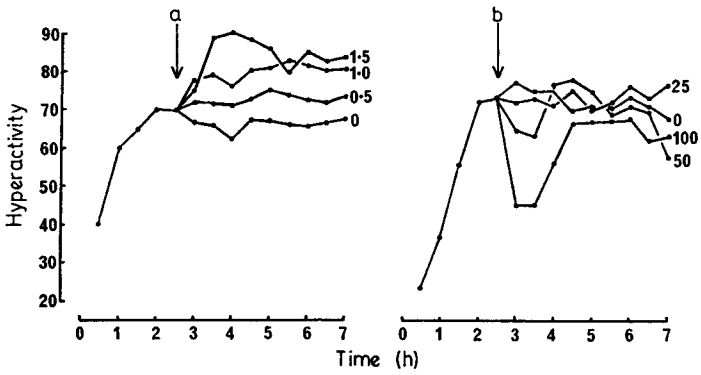

FIG. 2. Modification by a-(+)-amphetamine and b-piribedil of the hyperactivity induced by 50 μg μl^{-1} dopamine administered into the nucleus accumbens 2 h after pretreatment with nialamide (100 mg kg^{-1}, i.p.). Both amphetamine and piribedil were administered by the intraperitoneal route 2·5 h after dopamine (↓). Hyperactivity is expressed in counts per 5 min. 6–10 rats were used at each dose level of dopamine agonist which is indicated in mg kg^{-1}. Standard errors on the means are less than 18%.

careful observation of the rats showed that they were quiet at times when they were not biting. Bromocriptine antagonized the hyperactivity response (Fig. 1) but only at doses which may be considered very large in behavioural terms ($P<0{\cdot}001$ at 50 mg kg^{-1}) (see Johnson, Loew & Vigouret, 1976). During the first 60 min in which activity counts were reduced by bromocriptine (50 mg kg^{-1}, i.p.), the animals were not stereotyped and the reduction in response at this time may be considered to be a genuine depression of activity, but after this time all animals exhibited stereotyped biting. Piribedil caused very little change in the dopamine response; only the reduction recorded using a large dose of 100 mg kg^{-1} achieved significance ($P<0{\cdot}01$) (Fig. 2). In contrast to all other agents, amphetamine (0·5–1·5 mg kg^{-1}, i.p.) enhanced the dopamine response ($P<0{\cdot}001$ at 1·5 mg kg^{-1}) (Fig. 2).

Apomorphine (6·25–25 μg, in nitrogen-bubbled distilled water) administered bilaterally into the nucleus accumbens caused highly significant reductions ($P<0{\cdot}001$) in the dopamine response. The nature and time course of this effect was similar to that recorded after peripheral administration (Fig. 3). However, although (−)-NPA is a far more potent dopamine agonist as judged from the effects of peripheral administrations, and by its action in the nucleus accumbens in the absence of dopamine (Costall & others, 1975b), this agent caused only a modest reduction in the dopamine response at comparatively larger doses ($P<0{\cdot}05$ at 12·5 μg) (Fig. 3). The ability of (−)-NPA to induce biting following injection into the nucleus accumbens precluded the use of larger doses (Costall & others, 1975b). Previous studies have shown that, although apomorphine lacks the ability to induce hyperactivity from the nucleus accumbens when injected alone (Pijnenburg & others, 1976), (−)-NPA does cause a modest response (Costall & others, 1975a). However, the injection of (−)-NPA into the nucleus accumbens following lower doses of dopamine failed to increase the activity. Piribedil injected into the nucleus accumbens failed to significantly modify the dopamine response ($P>0{\cdot}05$) (Fig. 3) whilst amphetamine enhanced the activity ($P<0{\cdot}01$–$P<0{\cdot}001$ at 25 and 50 μg) (Fig. 3). This was more marked at a submaximal dose of dopamine (6·25 μg).

Of the dopamine agonists tested, apomorphine was shown to be the only one to antagonize the dopamine response in substereotypic doses administered peripherally, and was the only agent to antagonize the dopamine response on direct injection into the nucleus accumbens. This may simply indicate that whilst apomorphine has affinity for the dopamine receptors in the nucleus accumbens, it may lack intrinsic activity. That the aporphines may possess mixed agonist/antagonist properties is indicated by their ability to cause both an activation of striatal adenylate cyclase and to antagonize a dopamine stimulation of this enzyme system (Miller, Kelly & Neumeyer, 1976). This agrees with observations that neuroleptic agents also antagonize dopamine stimulated adenylate cyclase and similarly inhibit a dopamine-induced hyperactivity from the nucleus accumbens (Costall & Naylor, 1976). Clearly, for direct correlation to the present observations, it is necessary to study changes in the activity of adenylate cyclase from the nucleus accumbens but, nevertheless, these findings may be pertinent. Alternatively, it is tempting to speculate on the relation of the observed antagonism to an action of apomorphine on 5-HT mechanisms in the nucleus accumbens (Grabowska, Antkiewicz & others, 1973). It has been shown that 5-HT may modulate the dopamine hyperactivity such that small amounts of 5-HT injected into the nucleus accumbens after dopamine will, similarly to apomorphine, antagonize the dopamine response (Costall, Marsden & others, 1976).

Nevertheless, whatever the mechanisms involved

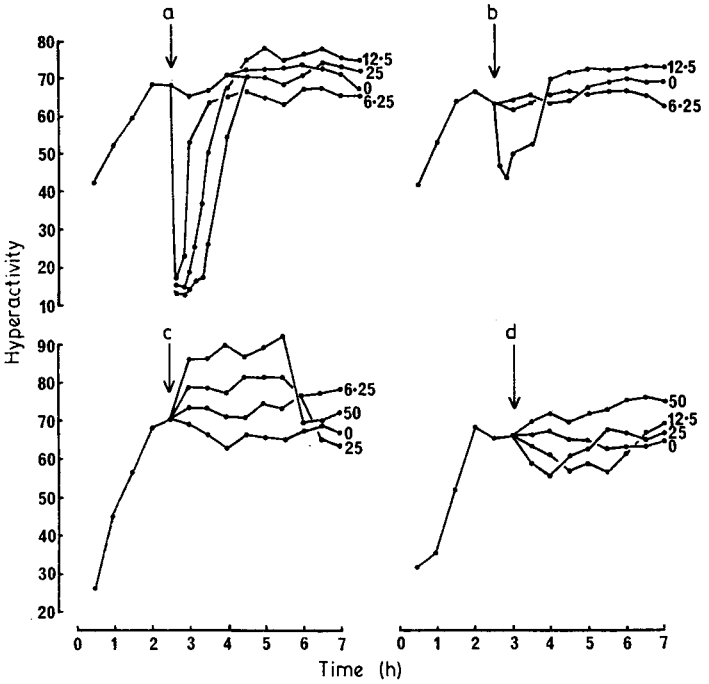

FIG. 3. Modification by a-apomorphine, b-(—)-NPA, c-(+)-amphetamine and d-piribedil of the hyperactivity induced by 50 μg μl^{-1} dopamine administered into the nucleus accumbens 2 h after pretreatment with nialamide (100 mg kg^{-1}, i.p.). The dopamine agonists were administered directly into the nucleus accumbens in a volume of 1 μl 2·5 h after dopamine (↓). Hyperactivity is expressed in counts per 5 min. 6–10 rats were used at each dose level of dopamine agonist which is indicated in μg. Standard errors are less than 12% of the means.

with the action of apomorphine in the nucleus accumbens, it is clear that this agent which is virtually *the* classical dopamine agonist is most probably not an agonist at those dopamine receptors in the nucleus accumbens which control locomotor activity.

This work was supported by the Medical Research Council. Miss M. Y. Green gave excellent technical assistance. Professor J. L. Neumeyer kindly provided (—)-NPA. Gifts of drugs were also received from Sandoz and Servier.

January 27, 1976

REFERENCES

COSTALL, B. & NAYLOR, R. J. (1975). *Eur. J. Pharmac.*, **32**, 87–92.
COSTALL, B. & NAYLOR, R. J. (1976). *Ibid.*, **35**, 161–168.
COSTALL, B., MARSDEN, C. D., NAYLOR, R. J. & PYCOCK, C. J. (1976). *J. Pharm. Pharmac.*, **28**, 248–249.
COSTALL, B., NAYLOR, R. J. & NEUMEYER, J. L. (1975a). *Ibid.*, **27**, 875–877.
COSTALL, B., NAYLOR, R. J. & NEUMEYER, J. L. (1975b). *Eur. J. Pharmac.*, **31**, 1–16.
COSTALL, B., NAYLOR, R. J. & PINDER, R. M. (1976). *Psychopharmacologia (Berl.)*, in the press.
DE GROOT, J. (1959). *Verh. K. Ned. Akad. Wet.*, **52**, 14–39.
ELKHAWAD, A. O. & WOODRUFF, G. N. (1975). *Br. J. Pharmac.*, **54**, 107–114.
GRABOWSKA, M., ANTKIEWICZ, L., MAJ, J. & MICHALUK, J. (1973). *Pol. J. Pharmac. Pharm.*, **25**, 29–39.
IVERSEN, S. D., KELLY, P. H., MILLER, R. J. & SEVIOUR, P. (1975). *Br. J. Pharmac.*, **54**, 244P.
JOHNSON, A. M., LOEW, D. M. & VIGOURET, J. M. (1976). *Ibid.*, **56**, 59–68.
KELLY, P. H. (1975). *Ibid.*, **55**, 291P.
KELLY, P. H., MILLER, R. J. & NEUMEYER, J. L. (1975). *Ibid.*, **54**, 271P.
KELLY, P. H., SEVIOUR, P. W. & IVERSEN, S. D. (1975). *Brain Res.*, **94**, 507–522.
MILLER, R. J., KELLY, P. H. & NEUMEYER, J. L. (1976). *Eur. J. Pharmac.*, **35**, 77–83.
PIJNENBURG, A. J. J. & VAN ROSSUM, J. M. (1973). *J. Pharm. Pharmac.*, **25**, 1003–1005.
PIJNENBURG, A. J. J., HONIG, W. M. M., VAN DER HEYDEN, J. A. M. & VAN ROSSUM, J. M. (1976). *Eur. J. Pharmac.*, **35**, 45–58.
PIJNENBURG, A. J. J., HONIG, W. M. M. & VAN ROSSUM, J. M. (1975). *Psychopharmacologia (Berl.)*, **41**, 175–180.